

A national scheme using digital images of blood cell morphology to support continuous professional development: Evaluating morphology reporting

By

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Declaration

I declare that whilst registered as a candidate for the Doctorate in Biomedical Science at the University of Portsmouth I have not registered for any other research award at any other university. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other scientific award. The work contained within this submission is my own work and, to the best of my knowledge and belief, it contains no material previously published or written by another person except where due acknowledgement has been made in the text.

Abstract

The reporting of blood cell morphology, by biomedical scientists using microscopy, is a subjective and relatively uncontrolled process; morphology reports impact directly upon the clinical care of patients, however, no large studies of the processes morphologists employ to reach their conclusions have been undertaken.

This thesis chronicles the collaborative process with a national provider of quality assessment services (UK NEQAS(H)); detailing the pioneering developments that culminated in the creation of a national scheme, incorporating digital images of peripheral blood cells and accredited for continuous professional development. Annual exercises, using digital images distributed via the internet, were used to develop, test and create the scheme. Two workshops provided early assessment and feedback from participants. The aim of this research was to then evaluate the responses of the large number of professionals who completed cases, to give insights into how they interpret the blood cell morphology to produce their succinct report.

The responses of between 732 and 1,018 participants (median 878) were examined for five digital morphology cases specifically selected to cover a range of morphological features. The subsequent data examination shows that patterns of error and bias were found in the responses that have not been described in blood film reporting before. Where a single morphological abnormality existed (glandular fever or Pelger-Huët anomaly), the ability to identify the feature of interest was high (97% and 84% respectively), however, errors in knowledge-based classification were seen. For complex cases, with multiple abnormal features, additional errors of inattention and premature completion were found; in the case of lymphoma with oxidative haemolysis 68% correctly reported the acute haemolysis, however, only 17% correctly reported both abnormal pathologies. Heuristic methods of decision-making, not considered in morphology reporting before, help the understanding of these patterns of error and bias.

Following this research the national scheme will be adapted to support participants by indicating the potential common forms of error found in morphology reporting.

Words: 315

Contents:

Chapter One: Introduction.....	22
1.1 Professional practice and clinical governance in pathology laboratories.....	22
1.2 The modern haematology laboratory.....	23
1.2.1 Quality assurance within the haematology laboratory.....	24
1.2.2 Automating morphology examination.....	25
1.3 Challenges faced by laboratories providing blood cell morphology reporting	26
1.4 Examination and reporting of blood cell morphology.....	27
1.5 External quality assessment and the role of UK National External Quality Assessment Scheme for General Haematology.....	28
1.5.1 The UK NEQAS(H) scheme for blood cell and bone marrow morphology using glass slides.....	30
1.5.1.1 The glass slide scheme: operational issues.....	30
1.6 The emergence of internet based digital Images of blood cell morphology for laboratory professionals.....	32
1.6.1 Digital images: quality and governance.....	34
1.7 The digital morphology project in collaboration with UK NEQAS(H).....	35
1.7.1 Developing professional consensus and educational elements.....	36
1.7.2 Responding to participant requirements and changing technology.....	38
1.7.3 Assembling images to provide a larger field of view.....	40
1.8 Generating high quality images for digital morphology: technical challenges.....	44
1.8.1 Developing large images taken at high resolution.....	47
1.8.2 The role of images compared to glass slide blood films for morphology.....	50
1.9 Creation of the UK NEQAS(H) digital morphology library.....	55

1.10	The UK NEQAS(H) digital morphology pilot scheme: a two year trial.....	56
1.10.1	Lessons learnt from the UK NEQAS(H) digital morphology pilot scheme.....	59
1.11	Perceptions of image quality: workshop.....	60

Chapter Two: The UK NEQAS(H) digital morphology scheme for continuing professional development.....64

2.1	Selecting equipment for the UK NEQAS(H) digital morphology project.....	64
2.1.1	Selection of microscope and camera system.....	64
2.2	Optimising the image.....	68
2.3	Demands for the virtual slide: stitching images.....	70
2.4	Expectations of the image from potential participants.....	71
2.4.1	Manipulation of the image: adding and removing features.....	72
2.5	Criteria for case selection.....	75
2.6	The requirement for secure and robust data management.....	77
2.7	Challenges designing the question format.....	78
2.7.1	Technical development of an electronic question format.....	78
2.7.2	Setting the questions: clinical and scientific relevance.....	81
2.8	Defining the feedback mechanism.....	82
2.8.1	Preparing the narration and annotations.....	82
2.8.2	Professional validation mechanisms.....	85
2.9	Implementation of the UK NEQAS(H) digital morphology scheme for CPD.....	85
2.10	Early evaluation of the UK NEQAS(H) digital morphology scheme for CPD.....	89
2.10.1	Participant feedback at the end of year one.....	89

2.10.2	Workshop at the end of year two.....	91
2.11	Examining a stitched image: the viewing experience	95
2.11.1	Examining a stitched image: actual practice.....	96
2.12	The UK NEQAS(H) digital morphology scheme for CPD at 4 years: proposal to examine participant responses.....	103
2.12.1	Current status and future requirements.....	103
2.12.2	Evaluating the reporting process to inform future direction.....	103
2.13	Proposal to evaluate participant data to test the association between feature selection and report outcome.....	104
2.13.1	The questions for my research	106
2.13.2	Ethical considerations and governance of research.....	106

Chapter Three: Methods used to evaluate participant responses.....107

3.1	Selection of digital morphology cases for data examination.....	107
3.2	Data extraction, coding and methods of analysis.....	108
3.2.1	Coding morphological features.....	109
3.2.2	Coding the multiple choice question.....	110
3.2.3	Coding the free text interpretive comments: morphological diagnosis	111
3.3	Limitations of data analysis.....	112
3.3.1	Professional skill level for morphology reporting of participants.....	112
3.3.2	Lack of trial conditions.....	113
3.3.3	Bias at coding of data	114

Chapter Four: Results with interpretive comment.....115

4.1	Single morphological feature Case 1: viral infection (EBV causing glandular fever)...	115
4.1.1	Morphological feature selection.....	117
4.1.2	Free text diagnosis question.....	117
4.1.3	Action selected.....	119
4.1.4	Case 1: summary findings from data.....	122
4.2	Single morphological feature Case 2: Pelger-Huët anomaly.....	123
4.2.1	Morphological feature selection.....	125
4.2.2	Free text diagnosis question.....	126
4.2.3	Action selected.....	128
4.2.4	Case 2: summary findings from data.....	130
4.3	Complex morphology Case 3: Microangiopathic haemolysis and viral infection.....	133
4.3.1	Morphological feature selection.....	134
4.3.2	Free text diagnosis question.....	135
4.3.3	Action selected.....	137
4.3.4	Case 3: summary findings from data.....	137
4.4	Complex morphology Case 4: Haemoglobinopathy and acute leukaemia.....	141
4.4.1	Morphological feature selection.....	143
4.4.2	Free text diagnosis question.....	143
4.4.3	Action selected.....	145
4.4.4	Case 4: summary findings from data.....	146
4.5	Complex morphology Case 5: skill levels known	149
4.5.1	Morphological feature selection.....	151
4.5.2	Free text diagnosis question.....	151
4.5.3	Skill level selected.....	152

4.5.4 Case 5: summary findings from data.....	155
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Chapter Five: Discussion.....159

5.1 Cases with a predominant single morphological feature.....	159
5.2 Cases combining complex morphological features.....	161
5.3 Effect of experience on morphological reporting skills.....	163
5.4 Understanding the process of providing a morphology report.....	164
5.5 Reducing the effort when associating observations in context, consideration of heuristic techniques.....	165
5.6 Heuristic techniques in morphology reporting.....	166
5.7 Digital images and morphology education beyond the UK NEQAS(H) scheme.....	177

Chapter Six: Conclusion.....179

6.1 Research in context.....	181
6.2 Future work.....	183

Chapter Seven: Reflection.....183

References.....186

Appendices

Appendix A UK NEQAS(H) ethical form for sample collection.....	195
Appendix B Letter of Ethical Approval and Governance.....	196
Appendix C DM scheme cases from launch 2008 to March 2014.....	200
Appendix D Codes for morphology feature selections available to participants.....	202

Tables:

Table 1.1	Location of registrants completing cases in the DM pilot scheme.....	58
Table 3.1	Basic details of cases selected for examination.....	108
Table 4.1	Variety of morphological features selected by cell lineage.....	117
Table 4.2	Summary of morphological diagnosis grouped by overall conclusion reported.....	118
Table 4.3	MCQ: Participants preferred action.....	120
Table 4.4	Actions taken for each morphological diagnosis summary group.....	121
Table 4.5	Variety of morphological features selected by cell lineage.....	125
Table 4.6	Summary of morphological diagnosis grouped by overall conclusion reported and showing participants ranking Pelger neutrophils as rank 1.....	126
Table 4.7	MCQ: Participants preferred action.....	128
Table 4.8	Actions taken for each morphological diagnosis summary group.....	129
Table 4.9	Variety of morphological features selected by cell lineage.....	134
Table 4.10	Top features selected by priority for each major cell lineage.....	135
Table 4.11	Summary of morphological diagnosis grouped by overall conclusion reported.....	136
Table 4.12	MCQ: Participants preferred action.....	137
Table 4.13	Variety of morphological features selected by cell lineage.....	143
Table 4.14	Top features selected by priority for each major cell lineage.....	143
Table 4.15	Summary of morphological diagnosis grouped by overall conclusion reported.....	144
Table 4.16	MCQ: Participants preferred action.....	146

Table 4.17	Variety of morphological features selected by cell lineage.....	151
Table 4.18	Top features selected by priority for each major cell lineage.....	151
Table 4.19	MCQ: Participants self-selected skill level.....	152
Table 4.20	Diagnostic summary by self-selected skill level.....	154
Table 4.21	Summary data overview from each case examined.....	158
Table 5.1	A seven-stage model to understand how professionals arrive at a final morphology report.....	165
Table 5.2	Summary of the processes used by participants enabling them to complete a morphological report shown in four panels	
	Panel A. Classification heuristics.....	169
	Panel B. Simplification heuristics.....	170
	Panel C. Context heuristics.....	171
	Panel D. Completion heuristics.....	172
Table 5.3	Morphology reporting skills and the types of error or bias shown in the five cases studied.....	175

Figures:

Figure 1.1	Example image from the first DM exercise for UK NEQAS(H) participants undertaken in 2000, as reported back at the symposium in October 2001.....	37
Figure 1.2	Example of feedback to UK NEQAS(H) participants following a DM exercise in 2003.....	39
Figure 1.3	Morphological features identified for participants.....	40
Figure 1.4	Creating a stitched image: Panels A and B.....	42
Figure 1.4	Creating a stitched image: Panels C and D.....	43
Figure 1.5	Digital image of a neutrophil showing typical granulation taken using a x100 objective lens.....	46
Figure 1.6	Sequential images taken at high resolution (x60 oil objective) to create a large image or “virtual”slide and the resulting ability to “zoom” to high magnification using QuickTime™	49
Figure 1.7	Example images from the 2004 UK NEQAS(H) DM exercise as reported back at the annual symposium.....	51
Figure 1.8	Comparison of morphological features selected from cases as reported from the conventional glass slide scheme and from the DM exercise.....	52
Figure 1.9	Participant opinion as to whether the 40 field images were of adequate size for reporting purposes.....	54
Figure 1.10	Participants responses to subjective questions following completion of the DM exercise.....	54
Figure 1.11	Screen shot from the digital image morphology library prepared for UK NEQAS(H).....	56
Figure 1.12	Screen shot of a library case from the pilot scheme.....	59

Figure 1.13	Examples of images taken from two cases stained by different laboratories.....	62
Figure 1.14	Images taken from the same case (sickle cell anaemia) but stained in different laboratories using different staining protocols.....	63
Figure 2.1	Snapshot comparative images from three different imaging systems used on four different cases.....	67
Figure 2.2	Photographs of the Zeiss Axio M1 imager system used for the creation of images for the UK NEQAS(H) DM project.....	68
Figure 2.3	Creating a large stitched image from sequential high power fields	71
Figure 2.4	Large image created by stitching eight rows of eleven single sequential images.....	73
Figure 2.5	Magnified section of image pre manipulation (Panel A).....	74
	post manipulation (Panel B).....	74
Figure 2.6	Decision Tree.....	79
Figure 2.7	Diagrammatic flow of how the scheme operates as viewed by a participant.....	84
Figure 2.8	UK NEQAS(H) leaflet announcing the launch of the DM scheme for CPD	85
Figure 2.9	Composite snapshot of an actual DM case from the scheme.....	87
Figure 2.10	Snapshots from Case 5 (UK NEQAS(H) DM scheme (1204DM). Panels A and B.....	88
Figure 2.11	Responders statement on the frequency of their film reporting.....	91
Figure 2.12	Usefulness of narrative as scored by workshop responders.....	92
Figure 2.13	Improved interest in morphology as scored by workshop responders..	93

Figure 2.14	“Do you see the main value of the DM scheme to be maintaining morphology skills?” as scored by workshop responders.....	94
Figure 2.15	“What is your preferred type of MCQ from the three most used at this point?”	94
Figure 2.16	User A: Heat map and the parasites most viewed. Outcome correct identification of <i>P. ovale</i>	98
Figure 2.17	User B: Heat map and the parasites most viewed. Outcome incorrect identification of <i>P. vivax</i>	99
Figure 2.18	User C: Heat map showing areas viewed with at least eight key features examined (areas in yellow). Outcome incorrect identification of <i>P. falciparum</i>	100
Figure 2.19	Comparison outcomes of species of malaria selected by different professionals viewing a case of <i>P. ovale</i> in different formats.....	101
Figure 2.20	User outcomes for Burkitt’s lymphoma image at the ISLH congress Australia 2008.....	102
Figure 2.21	Governance of research.....	106
Figure 4.1	Case 1: viral infection (EBV causing glandular fever).....	115
Figure 4.2	Case 2: inherited Pelger-Huët anomaly.....	123
Figure 4.3	Analysis of participant responses from the two cases with a single morphological feature. Panels A to D.....	131
Figure 4.3	Analysis of participant responses from the two cases with a single morphological feature Panels E and F.....	132
Figure 4.4	Case 3: Microangiopathic haemolysis with reactive lymphocytes of a viral infection.....	133

Figure 4.5	Analysis of participant responses in a complex case with related morphological features. Case 3: Microangiopathic haemolytic anaemia associated with viral illness (acute HIV infection). Panel A....	139
Figure 4.5	Analysis of participant responses in a complex case with related morphological features. Case 3: Microangiopathic haemolytic anaemia associated with viral illness (acute HIV infection). Panels B to E.....	140
Figure 4.6	Case 4: Haemoglobin SC disease with acute myeloid leukaemia.....	141
Figure 4.7	Case 4: Analysis of participant responses where morphological features reflected two separate unrelated pathological disorders (HbSC disease with AML). Panel A.....	147
Figure 4.7	Case 4: Analysis of participant responses where morphological features reflected two separate unrelated pathological disorders (HbSC disease with AML). Panels B to E.....	148
Figure 4.8	Case 5: oxidative haemolysis (G6PD deficiency) and adult T-cell leukaemia/lymphoma.....	149
Figure 4.9	Case 5: Analysis of reported features according to the self-selected skill level of participants, where the morphological features represented two separate pathological disorders (adult T-cell leukaemia/lymphoma and acute oxidative haemolysis). Panels A to D.....	156
Figure 4.9	Case 5: Analysis of reported features according to the self-selected skill level of participants, where the morphological features represented two separate pathological disorders (adult T-cell leukaemia/lymphoma and acute oxidative haemolysis). Panel E.....	157

Abbreviations:

Abbreviation	Definition
AML	Acute Myeloid Leukaemia
APML	Acute Promyelocytic Leukaemia
APO	Apochromatic
ATLL	Adult T-cell Leukaemia/Lymphoma
B cell PLL	B-Cell Prolymphocytic Leukaemia
BF	Blood Film code used for glass slide scheme
BMS	Biomedical Scientist
BSH	British Society of Haematology
CCD	Charge-Coupled Device
CGL	Chronic Granulocytic (Myeloid) Leukaemia
CLL	Chronic Lymphocytic Leukaemia
CMFT	Central Manchester University Hospitals Foundation Trust
CPD	Continuing Professional Development
CS	Creative Suite
DH	Department of Health
DM	Digital Morphology
DN	Data Number
EBV	Epstein Barr Virus
EQA	External Quality Assessment
FBC	Full Blood Count
FTP	File Transfer Protocol
G6PD	Glucose-6-Phosphate Dehydrogenase
Hb	Haemoglobin
HbSS	Homozygous Sickle Cell Haemoglobin
HCPC	Health and Care Professionals Council
HIV	Human Immunodeficiency Virus
IBMS	Institute of Biomedical Science
ICSH	International Committee for Standardization in Haematology
INCTR	International Network for Cancer Treatment and Research

IQC	Internal Quality Control
ISLH	International Society for Laboratory Hematology
ISO	International Organisation for Standardization
IT	Information Technology continuous monitoring systems
MAHA	Microangiopathic Haemolytic Anaemia
MDS	Myelodysplastic Syndrome
Medic	Medical officer/Clinical Haematologist
MCQ	Multiple Choice Question
NHS	National Health Service
NR-BMS	Biomedical Scientist non-reporter of blood films
NQAAP	National Quality Assurance Advisory Panel
PA	Code used for glass slide parasite scheme
PC	Personal Computer
<i>P. falciparum</i>	Malaria species <i>Plasmodium falciparum</i>
<i>P. ovale</i>	Malaria species <i>Plasmodium ovale</i>
<i>P. vivax</i>	Malaria species <i>Plasmodium vivax</i>
RCPATH	Royal College of Pathologists
RCPAQAP	Royal College of Pathologists of Australasia Quality Assurance Program
RDT	Rapid Diagnostic Test
R-BMS	Biomedical Scientist issuing unsupervised blood film reports
SAG	Scientific Advisory Group
SD	Standard Deviation
SEM	Standard Error of Mean
S-BMS	Biomedical Scientist issuing supervised blood film reports
TM	Trade Mark
TTP	Thrombotic Thrombocytopenic Purpura
UKAS	United Kingdom Accreditation Service
µm	Micrometer
UK NEQAS	United Kingdom National External Quality Assessment Service
UK NEQAS(H)	United Kingdom National External Quality Assessment Service for General Haematology

v	Version
WBC	White Blood Cell
WHO	World Health Organisation
XML	Extensible Markup Language
24/7	24 hours per day and 7 days per week

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Report from the UK NEQAS(H) on a web based pilot scheme for digital morphology. At the XXth 2007. International Symposium on Technological Innovations in Laboratory Hematology. 11th May 2007. Miami, USA.

Digital Morphology: the UK NEQAS(H) experience. Conference exercise and feedback. At the XXIst International Symposium on Technological Innovations in Laboratory Hematology. 1st May 2008. Sydney, Australia. (Joint with John Burthem).

Digital Morphology: A new UK NEQAS(H) scheme for continuous professional development. At the XXIInd International Symposium on Technological Innovations in Laboratory Hematology. 14th May 2009, Las Vegas, USA.

A digital morphology educational EQA scheme. At the European Committee for External Quality Assurance, Programmes in Laboratory Medicine. 22nd September 2011. Szeged, Hungary.

Digital Morphology: four years experience of the UK NEQAS(H) scheme for laboratory based professionals. At the XXVth International Symposium on Technological Innovations in Laboratory Hematology. 24th May 2012. Nice, France.

The UK NEQAS(H) scheme for Digital Morphology: Four years experience. At the National Clinical Laboratory Congress of China (NCCL). 23rd August 2012. Beijing, China.

Manuscripts:

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Brereton, M. L., De la Salle, B., Burthem, J., Ardern, J., Hickman, L., Seal, L., McTaggart, P., West, M., Swirsky, D., Parker-Williams, J., & Hyde, K. (2008). Review of the UK NEQAS(H) digital morphology pilot scheme for continuing professional development accessed via the internet. *International Journal of Laboratory Hematology*, 30(5), 365-371.

Relating to my research on participant responses to the digital morphology cases.

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Oral presentations at international meetings

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Manuscript:

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Consideration of Ethics and Governance

08/06/2011 Project Proposal Professional Doctorate Candidate 381533.

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The UK NEQAS scheme for General Haematology has overall governance for the Digital Morphology Scheme. This project was initiated in 2000 and is reviewed by the national Scientific Advisory Panel at the annual meeting of the Morphology Scientific Advisory Group. The overall development of the Digital Morphology scheme is governed by UK NEQAS (H) of which this project forms part. Ethical issues have been considered by both the Morphology SAG and UK NEQAS (H) under two main categories:

a/ The preparation of morphology cases is covered by the policies applied to the collection of all material for EQA purposes in that samples may only be used if they are discard material from routine testing which has been fully anonymised and is not traceable back to a patient. For the use of images in the digital morphology scheme clinical details and patient demographic data are also altered from the original, ie patient ages and test results are altered from the original source data.

b/ The extraction of participant input from the CPD scheme is totally anonymised and not traceable. It is not possible to associate extracted data to any individual. No participant demographic data will be extracted nor made available. This project will use only the morphology features selected and the diagnosis input. Once data is extracted, it will not be possible to associate data with any individual. The participants are not being tested, this data extraction is to interrogate the scheme design and ensure it is meeting objectives.



Professor Keith Hyde PhD, FIBMS, FRCPath

Director United Kingdom External Quality Assessment Scheme for
General Haematology [UK NEQAS(H)] &
Director World Health Organisation (WHO)
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**Clinical Director (Joint) Greater Manchester Laboratory Medicine
(Pathology) Network**

Consultant Clinical Scientist

Manchester Royal Infirmary Medical Centre

**Governance of ethical considerations of research controlled by UK NEQAS(H) no
further applications for ethics required.**

Chapter One: Introduction

The Department of Health's (DH) requirement for a publicly accountable National Health Service (NHS) has produced a number of important reports that directly influence the way pathology services are implemented and operated (HBN 15, 2005). Government led initiatives, including the review by Lord Carter of Coles on unwarranted variation in the operational productivity and performance of NHS acute hospitals in England, (Carter, 2016, page 36) demand that laboratories constantly strive to improve efficiency. They must maintain and improve quality standards in a patient focused environment. As laboratories evolve to meet these objectives they must embrace a culture of accountability and responsibility by effectively demonstrating to patients and other service users that they are continually achieving targets and tackling areas of weakness.

1.1 Professional practice and clinical governance in pathology laboratories

The Health and Care Professions Council (HCPC) were created by legislation; Health and Social Work Professions Order 2001, updated 2016 (Bircham Dyson Bell, 2016). The HCPC are the independent regulator of health professionals working within the NHS in the United Kingdom (UK) with the aim of protecting the public by setting standards and maintaining a public register of properly qualified professionals; including laboratory-based biomedical scientists (BMS) who meet those standards. The HCPC take action to prevent any individual on their Register continuing to practice should they be proven to be in breach of those standards (HCPC, 2014).

In January 2006 the HCPC introduced legislation requiring all professional registrants to take responsibility for proving their commitment to continuing professional development (CPD). This legislation, updated in 2011, aims to ensure that any individual who wishes to remain on the register demonstrates they are continuing to develop their knowledge and skills (HCPC, 2011). Importantly both the individual professional, and the laboratory that employs them, are subject to formal inspection by a number of national regulatory bodies including the UK Accreditation Service (UKAS). Both the individual and the organisation must provide evidence of compliance with professional standards and a commitment to raising skill levels by CPD, all within

a climate of increasing productivity and improving efficiency. Medical laboratories work to the international quality management standard developed by the International Organization for Standardization's Technical Committee 2012: ISO 15189. (ISO, 2012). Inspection of evidence against the standard is carried out by UKAS for all aspects of sample processing and result reporting (UKAS, 2017).

The Institute of Biomedical Science (IBMS) is the national professional body for biomedical scientists (BMS) who provide most of the technical services within routine service pathology laboratories. The IBMS publishes policies for professional practice (IBMS, 2017), sets examinations, offers professional educational facilities and provides a forum for laboratory staff to interact, however, membership of the IBMS is not mandatory but those who choose not to join must also comply with HCPC regulations and provide evidence of CPD but do so independently.

1.2 The modern haematology laboratory

The individuals' requirement to demonstrate CPD and improving skills have to occur in laboratories evolving their services to meet the public demand to access pathology whenever they require. The implementation of Agenda for Change in 2004 began the standardisation in the terms and conditions of service within the NHS (NHS Employers, 2016). This included rates of pay, so laboratories were encouraged to move away from minimal, but expensive, emergency style cover outside of core hours to a more robust continuous service. This new 24/7 laboratory also needed to provide a wider test repertoire than had generally been made available outside core hours at nights and weekends, requiring managers to replace minimal out of hours staffing with new shift patterns.

Laboratories are required to demonstrate that they provide high quality services, no matter when they are accessed, whilst remaining efficient and providing value for money. Managers are challenged to increase productivity, absorb increasing workloads, achieve faster turn-a-round times and ensure consistency so have invested in automated processes. This has led to an increased reliance upon the latest technology, including computer-based information strategies (IT) and also by workforce restructuring to reduce staff costs.

1.2.1 Quality assurance within the haematology laboratory

To achieve targets laboratories use sophisticated analysers to produce precise measurements of multiple biological parameters at a rapid speed for immediate clinical use. To achieve speed the analysers are designed to detect and measure analytes within predefined limits into which the majority of patient results are expected to fall. Such analysers are subject to intensive validation and verification processes, and employ rule-based, continuous IT monitoring systems to ensure the results produced are rigorously controlled to the highest possible quality standard. For the haematology laboratory, the main routine test profile is the full blood count (FBC) which provides a measure of the quantity and quality of all the cells expected to be present in peripheral blood (Brereton, McCafferty, Marsden, Kawai, Etzell & Ermens, 2016). The FBC is processed using highly automated analysers, with flow cytometry technology to produce a profile of at least 20 different test parameters from a single sample in a matter of minutes. Individual laboratories may analyse over a thousand individual patient FBC samples every day so must run their analysers to the highest level of precision and accuracy and be able to prove to external inspectors that they are doing so.

Where available, biological material of a known data value is used as a calibrator to ensure those tests are as accurate as possible when referenced against this value. Analyser manufacturers also produce material of set values termed internal quality control (IQC) to check analyser function; laboratories then define a regular protocol for IQC use. Furthermore, for high volume testing of patient samples, the laboratory can set limits in the analyser software against all component tests of the FBC and then monitor the mean values obtained from batches of patient results giving a real time measure of the precision of analyser function. Should the quality of the results deteriorate, or values drift beyond those preset limits the analyser will stop. A BMS must then investigate the cause before any more patient samples can be processed.

Despite such advances in the testing and control of the FBC, the any subsequent examination of that sample generated results which lie outside the set limits, is usually carried out manually via the labour intensive examination of a blood film. These blood films are prepared by spreading a drop of blood of variable size ($< 5 \mu\text{l}$) on a glass slide

and staining the smear using a combination of biochemical stains with acidic and basic dyes reliant on the pH of cellular components (Horobin & Walter, 1987). The stained slide is then examined by an experienced laboratory-based BMS using microscopy. Even in the modern haematology laboratory the examination and reporting of the blood film remains primarily a manual and, therefore, highly subjective process (Constantino, 2015). Demonstrating effective IQC for morphology reporting is difficult, laboratories should introduce some form of competence testing for their staff but there are no guidelines upon which to base the criteria for this (Sinclair, 2005). The blood film report issued by the BMS may drive further test selection and provides key diagnostic information to the clinician, upon which action will be taken and medical treatments influenced or determined.

1.2.2 Automating morphology examination

Semi-automated cell recognition systems are available and successfully used in some laboratories; the most widespread in the UK being produced by the Swedish based company CellaVision™ (Ceelie, Dinkelaar & Gelder, 2007). Originally designed to use a standard microscope these systems scan the blood film to locate possible white cells then produce a series of separate images of individual white cells taken with x50 or x100 oil immersion objective. The images are then presented in a probable differential classification for a BMS to verify as correct or re-classify the cells (Brereton *et al.*, 2008c; Rezaatofghi & Soltanian-Zadeh, 2011). The advantages to such systems include the speed in which they can produce a white cell differential, that images can be viewed by a number of people locally and remotely and the fact that results can be stored and are auditable. The systems are less advanced for red cell imaging, possibly because the lack of an internal structure *e.g.* granules or nucleus, means the system has no textural point to focus on, making red cells less easy to capture effectively. The technology requires complex IT interfacing to be used within the automated workflow, these reasons may explain why uptake nationally has been slow. Additionally, in the UK, the laboratory cannot alter the recognition capability, so if the system is poor at classifying any particular cell type it cannot be improved and user irritation can occur knowing the system will continually fail to correctly classify certain cells. The blood film limits the number of cells these systems can be set to classify, usually 100 to 200 white

cells, however, the automated FBC analysers process a larger volume of sample and classify more than 5,000 cells to produce a white cell differential (Meintker, Ringwald, Rauh & Krause, 2013). So, providing abnormal cells are not detected the BMS will usually select the results from the FBC analyser in preference. There are many reasons for examining a blood film, of which producing a white cell differential is a small part of the process. These considerations, along with the cost, cause laboratories to consider carefully whether they are appropriate.

Microscopic viewing of a blood film in order to examine blood cell morphology is still regarded the 'gold standard' for interpreting abnormal FBC results for the requesting clinician (Bain, 2005). To perform the microscopic examination effectively requires that the BMS has a high level of expertise and an extensive knowledge of haematology, yet there are no professional guidelines or proficiency tests set at a national level for this service. Laboratory protocols should dictate that abnormal blood films are referred to a Consultant Haematologist for interpretative clinical comment, although to do this the BMS must recognise that an abnormality is present and understand when an abnormality requires referral.

1.3 Challenges faced by laboratories providing blood cell morphology reporting

Providing evidence and assurance verifying blood cell morphology reporting, which relies upon subjective recognition and interpretative skills by individuals, sets laboratories a great challenge, and little has been published on the reporting process to support them.

The modern laboratory struggles to justify the labour intensive training and support previously used to raise skill levels for morphology reporting, where high levels of technical expertise are still required. Those BMS performing microscopy need to provide evidence for inspectors that they are maintaining and continuously developing their skills. This conundrum proves difficult to resolve, especially as morphology reporting expertise may be concentrated in a few key individuals, and presents a particular problem for smaller laboratories where the opportunity to gain experience of complex morphological cases may be limited. Supporting continuous 24/7 access to quality morphology reporting means that laboratory managers must provide training

and CPD opportunities; a problem when shift patterns dilute expertise from the day and so disrupt conventional training initiatives. With experienced staff working varied shifts across different sites it becomes difficult to arrange group based tutorials, allow staff time out of the laboratory to attend lectures, off-site training courses or to provide one to one training. One way to meet these demands is for employers and employees to embrace computer-based technologies and adopt new ways of supporting education and training (Kumar *et al.*, 2004).

1.4 Examination and reporting of blood cell morphology

Many haematology laboratories have an automated process for blood film preparation, where the smear is made by a mechanical device and then stained either by the same device, or by a separate “staining” machine. There is published guidance on staining methods both from the International Committee for Standardization in Haematology (ICSH) (ICSH, 1984) and in the nationally popular reference book Dacie and Lewis Practical Haematology (Lewis, Bain & Bates, 2008). Manufacturers of stains and staining machines also offer guidance (Sysmex, 2017). Each laboratory will, however, then design its own specific protocol, choose to prepare or buy pre-prepared stains and select their own preferred timings to be used by the staining procedure to create a final blood film for examination by microscopy. BMS will become familiar with the appearance of films from their own laboratory but this will differ across sites. Essentially this lack of a core standard introduces a challenge for those learning and then practicing morphology reporting (Rajamaki, 1980). Such a subjective process demands that the quality of the blood film being examined is optimum; however this may not be the case. BMS who work for large NHS Trusts may find themselves working their shifts at different laboratory locations and may even find variation in staining quality across sites.

BMS will have varied opportunities to acquire morphology reporting skills, their first experience being the training they receive at universities whilst obtaining their degree, but this is likely to cover only the basic techniques of using a microscope with a limited number of cases. Once employed, however, they will receive their initial exposure of microscopy in working practice and the opportunity to see a wide variety of different pathological disorders. Successful training requires that an expert or an experienced

BMS has time with the trainee morphologist, that they have an extensive selection of blood films showing all aspects of morphology they might encounter and access to reputable published training material (Bain, 2015).

Laboratories set their own criteria for defining at what point their BMS staff are competent to report patient blood films and define a process for referring clinically urgent and abnormal findings. Under ISO 15189 laboratories must also document their expectations of what a person might safely report outside routine service hours and what expert support is available. The Royal College of Pathologists (RCPATH) published a guideline for the communication of critical results but this is limited and not mandatory (RCPATH, 2015). The lack of national support for BMS reporting blood films remains largely unrecognised, it is only recently that the ICSH has published guidance for morphology nomenclature but these are unenforceable recommendations and do not provide offer guidance on the format of the final report (Palmer *et al.*, 2015).

When providing a morphology report the BMS must report the abnormal features they see, know how to react to those features and then decide what action to take. This might be to phone the clinical team, add further tests or to refer the film to a clinical colleague. It is also important that their report has meaning to the clinicians caring for the patient, who need to understand the significance of the features reported or their actions might not be appropriate. An experienced morphologist will summarise his or her morphology comments so as to convey an appropriate summary of their findings and may also suggest further tests or advise clinical referral to another team.

1.5 External quality assessment and the role of the UK national external quality assessment service for general haematology

Managing quality is an essential component of the daily processes for haematology services, although the real-time result monitoring using IQC and patient batch mean data (Chapter 1.2) is not easily applied to morphology reporting. Laboratories also subscribe to externally run quality assessment schemes (EQA) to compare the standard of their test results against that of other laboratories performing the same tests and using the same reagents or equipment. The UK National External Quality Assessment Service (UK NEQAS) is a company limited by guarantee (number 3012351) and a registered charity (number 1044013), the company vision is to use education as a tool

to improve standards in pathology laboratories, in doing so, to optimise patient care (UK NEQAS, 2017a). Any quality assessment scheme conforming to professional quality standards and complying with the UK NEQAS code of practice can be awarded a UK NEQAS designation. The main provider of EQA services for haematology laboratories in the UK is the UK NEQAS service for general haematology (H) located at Watford General Hospital.

EQA schemes distribute material, by post, to registered laboratories which perform the tests within a set time frame and send their results back to the scheme headquarters, usually electronically, for subsequent comparison analysis. The reports returned will compare the performance of the registered laboratory to the consensus of all participating laboratories and/or to an expert opinion and often provide a mathematical score of performance. Should the performance of a laboratory in a NEQAS scheme be substandard, it is the duty of the scheme's manager to report poor performance to the relevant laboratory manager and clinical lead. In the first instance UK NEQAS(H) seek to offer advice and to support laboratories with understanding why the poor performance has occurred and to resolve any technical issues which may be the root cause. If a laboratory has a persistent poor performance then UK NEQAS(H) must also alert the national quality assurance advisory panel (NQAAP) for haematology. This panel has the responsibility of ensuring standards for analytical and interpretative work in UK laboratories; the IBMS nominate a professional representative to sit on the panel alongside a representative from the RCPATH. The UK NEQAS(H) Steering Committee has an integrated relationship with the NQAAP, liaising over poor performance and reporting advice offered to participants with performance issues. Laboratories must disclose any poor performance letters they have received from UK NEQAS(H) to UKAS inspectors.

In the UK, participation in EQA schemes is voluntary; however laboratories are required to produce evidence of participation in EQA programmes to various inspection bodies. Many laboratories also provide services to clinical trials and commercial enterprises which require that the laboratory is not only certified by independent inspection (*e.g.* UKAS) but has proven good performance in registered EQA schemes.

1.5.1 The UK NEQAS(H) scheme for blood cell and bone marrow morphology using glass slides

EQA is available for morphology reporting in the UK, the most widely recognised scheme being the blood cell and bone marrow reporting scheme run by the UK NEQAS(H). This scheme began in 1968 and is popular, not only in the UK where more than 500 laboratories are registered including some from the private sector, but also from 80 non-UK countries where local national EQA schemes either do not provide an option for morphology reporting (*e.g.* Ireland) or have limited distributions of material (*e.g.* Portugal) (UK NEQAS, 2017b).

The operation and developments of the scheme are overseen by a scientific advisory group (SAG) comprising of professionals, both clinical and scientific, who have significant experience and expertise in morphology reporting. The morphology SAG meet annually to select and approve the glass slide peripheral blood films that will be used by the scheme for distribution to participating laboratories. In recent years they have also overseen developments such as the increase in number of surveys distributed annually to eight in 2015 and the introduction of electronic data return in 2016. The morphology SAG also reviews the schemes findings and the chair will report on developments and problems to the UK NEQAS(H) Steering Committee, comprised of the chairs of various haematology SAGs along with the manager and scheme director of UK NEQAS(H). In 2016 the morphology SAG looking to comply with ISO 15189 and reported some level of performance scoring would be developed for the glass slide scheme, to be introduced in 2018 (UK NEQAS(H), 2016).

As a member of the morphology SAG since 2006, understanding the operational aspects of this scheme, its aims, achievements and limitations was essential when considering the possibilities for employing digital imaging technology for blood cell morphology.

1.5.1.1 The glass slide scheme: operational issues

Each survey contains two morphology cases, in the form of Romanowsky stained blood films posted out to participating laboratories, making a total of 16 cases per annum. With hundreds of laboratories participating both in the UK and abroad UK NEQASH)

require at least 700 blood films prepared for each case. Laboratories have approximately 10 days to examine the blood films, they are asked to:

- Report up to 5 morphological features selected from a standard list of 72 options, there is no priority rank to those features.
- Suggest a morphological diagnosis

Providing glass slide prepared blood films is comparable with the way most laboratories examine and report blood films so is suitable for EQA purposes but by default there are limitations.

- To prepare a case there must be suitable blood available to make the sufficient blood films. Obtaining good material from unusual clinical cases or from children and babies is difficult to arrange.
- There are logistical challenges in preparing and sending slides to so many laboratories, both in the UK and beyond, also in both the time it takes for laboratories to receive the cases and the time allowed for queries or repeat slides to be sent has to be factored in to the process.
- There is the cost of preparing the material and the postage.
- The appearance of the stain will be different to the laboratories own stain.
- Registration and participation are by the laboratory as an entity and not by the individual professionals who are reporting the blood films, so the scheme cannot test an individual's proficiency.
- UK NEQAS(H) do not know whether slides are examined by one member of the laboratory staff, or reviewed by a team, neither is the level of experience known for the person reporting the slides. Without this information the scheme is limited about what scoring assessment can be offered and remains primarily educational into 2017.

The glass slide surveys can provide laboratories with a good educational resource once the survey is completed and the final reports received, however, this process takes several weeks making engagement with staff on shift patterns a challenge. The laboratory must also provide storage space for the glass slides and access to copies of the relevant reports. Glass slides require cleaning and ideally covering with a thin glass

piece to protect them from being scratched whilst in storage. Glass slides are easily damaged or may simply not be returned correctly to storage by multiple users thus reducing the value of the resource.

Whilst BMS reporting blood films do not provide a clinical diagnostic report for patients, as per regulation of practice by the HCPC, the morphological comments they do report are an interpretative summary of their findings and can directly impact on the clinical treatment or further testing that patients may then undergo. This infers that the BMS reporting films carry a high degree of responsibility for providing clinicians with the correct morphological information, yet there is no data on how influential that film report might be. In recognition of this situation UK NEQAS(H) actively encourage laboratories to suggest a morphological diagnosis for the glass slide survey cases. UK NEQAS(H) collate the returns and issue a report including histograms of consensus data of the morphological features selected and an expert opinion, which provides guidance for improved reporting and comment on the actual morphological diagnosis. The process takes time with reports available a number of weeks after the scheme has closed. Prior to 2014, reports were sent to the laboratory by post, they are now available electronically to laboratory leads, however, the mechanism by which reports are disseminated to the staff is undocumented. The length of the entire process creates difficulties with feedback to BMS whose shift patterns may mean that those receiving and reviewing the reports may not be those who originally participated in the survey.

Overall, with so many laboratories participating, the glass slide scheme remains extremely successful. A key limitation being that it does not provide a measure of competency in morphology reporting for participating laboratories nor does it provide an indication of the standards of reporting between individuals within laboratories.

1.6 The emergence of internet-based digital images of blood cell morphology for laboratory professionals

The emergence of digital imaging within service pathology laboratories was most noticeable in histopathology where the scanning of tissue sections, at acceptably low magnification (x40 objective or less), could be easily achieved relatively successfully for archive. The storage of images has, in some histopathology laboratories, replaced the

need to store tissue sections on glass slides whilst the production of images deemed of satisfactory quality for education and teaching has been achieved without major expense (Krippendorf & Lough 2005). The use of histopathology images for the actual reporting of patient specimens has developed, understandably with caution (Furness, 2007), but the drivers for employing images in a teaching environment for histopathology were clear as a limited amount of pathological material could be used and reused safely to teach numerous individuals.

The acceptance of images for blood cell morphology education at a professional level in haematology was originally met with more resistance; the reluctance to embrace imaging was because the resolution of images taken with a x40 objective provided insufficient cellular detail (Szu-Hee 2005). However there are many advantages for incorporating digital images into a professional educational process (Solberg, 2012):

- A single image can be evaluated by many users so multiple samples are not required.
- Every user evaluates the same material so there is no variation in content.
- Images are preselected for items of interest.
- Reduced exposure to pathological material improves health and safety.
- Image data is easily anonymised improving governance of patient information
- Access to rare cases and low volume material as only one original image is required.
- Potential to increase educational impact across geographic distance.
- User access can be away from the laboratory.
- Stability of image and retention of quality over time.
- Organisation of archive material with storage of information with the image.
- Ease and speed of retrieval from storage.
- Transportable.
- Annotation or narration against the image for education.
- Cheap to acquire, store and replace.

Convincing pathologists that images could be effective as a training tool for professionals who would then go on to report actual patient samples using microscopy

presented challenges. Digital technology provides an environment which is simply not the same as reporting using microscopy.

Unless the image is equivalent size as the original tissue the preparation of the image will inevitably focus on features that are meant to be found. This bias may impact on the user's ability to translate the learning experience to a clinical reporting environment where the significant features may be less easily found (Kumar *et al.*, 2004).

In other areas of pathology digital images had begun finding a role alongside actual glass slides as both a solution to the problems of physical storage and archive, and also as a tool for education and teaching of professional skills such as recognition and classification of cellular tissues (Leong, Graham, Schwarzmann & McGee, 2001; Steinberg & Ali, 2001; Dee, Lehman, Consoer, Leaven & Cohen, 2003). In laboratory haematology, however, the quality of the first widely published images failed to convince the professions that images could, in some areas, be a suitable alternative to the original blood or bone marrow smear kept on a glass slide (Szu-Hee, 2005).

1.6.1 Digital images: quality and governance

The global success of the internet as a tool for delivering information for teaching and education has enabled the publication of material from a myriad of sources, available for access by all, but often lacking formal peer review or validation by expert reference. The resultant explosion of material available enables individual professionals to access new resources in real time beyond the perceived restrictions associated with conventional sources via libraries or academic institutions. The use of digital cameras fitted on to microscopes saw a plethora of images appearing on the internet with little validation, examples of poor quality images, some with incorrect or misleading content are easily found.

Laboratories across the UK have witnessed the explosion of internet-based information over the past ten years, encouraging free or paid access to a wide spectrum of educational materials. Such materials are frequently produced without reference to source material, lack structured content and fail to respond to the requirements or

feedback of users. Importantly internet-based education material often lacks verification by expert professionals.

An internet-based resource that pertains to offer educational information to laboratory professionals must gain the trust of those individual professionals in order to maintain engagement and become successful. It is essential that the quality of the information provided is understood to be validated by appropriate experts and that the standard of the product is considered sufficiently high to meet the needs of the user (Luethi, Risch, Korte, Bader & Huber, 2004).

1.7 The digital morphology project in collaboration with UK NEQAS(H)

Close professional links between staff in laboratory haematology at Central Manchester University Hospitals NHS Foundation Trust (CMFT) and Manchester Universities had been in place for many years in order to deliver training and degree courses at the universities. With senior laboratory staff at CMFT also involved with the UK NEQAS(H) steering committee and SAGs the opportunity arose, in 2000, to form a collaborative partnership of interested professionals, with the aim of exploring possible uses for the emerging digital technologies as part of EQA for blood cell morphology. Project development was to be undertaken in Manchester with guidance, advice and support from UK NEQAS(H) headquarters at Watford. The digital morphology (DM) project would be subject to the ethical codes of practice of UK NEQAS(H) for sample collection and data handling. All decisions and developments would need authorisation from the UK NEQAS(H) morphology SAG and ultimately the steering committee.

It was not known whether laboratories would accept a role for digital morphology at a national level and, if so, what form that role would take. Importantly the principal driver for the direction of the project would be the needs of participants of the well-established glass slide scheme. This would be achieved by running a series of internet-based exercises and workshops for participants, the results of which were then fed back at the annual UK NEQAS(H) symposium. If the DM project was to lead to the development of a successful product it was imperative that the requirements and opinions of prospective users were fully incorporated into the project at all stages.

Another unknown would be how laboratories were going to respond to the influx of technologies at the start of the digital age, indeed many laboratories had limited internet access with personal computers (PCs) being a small but growing part of laboratory hardware.

The advantages that high quality digital images, the content of which has been verified by experts and delivered to users in a consistent and secure manner, have over conventional glass slide blood films for professional education purposes were acknowledged (Brueggeman, Swineheart, Yue, Conway-Klaassen & Wiesner, 2012). The key attraction for using images in education is that all participants accessing material are presented with exactly the same cells.

1.7.1 Developing professional consensus and educational elements

From 2000 the increasing use of computer-based technology within routine hospital laboratories enabled the DM project team at CMFT to develop a preliminary test exercise in collaboration with UK NEQAS(H). Using a Nikon E400 microscope with high quality x50 magnification plan apochromatic oil-immersion objective attached to a Nikon Coolpix 995™ camera and Coolpix relay lens (0.82-0.29) and Lexar™ card reader/writer (model GS-UFD-20SA-TP) a selection of five single frame images were taken of blood cells from two different blood films that UK NEQAS(H) had previously distributed as glass slide surveys (film one: chronic myelomonocytic leukaemia; film two: leucoerythroblastic film with disseminated intravascular coagulation). These 10 images were released to participating laboratories via the UK NEQAS(H) website. Laboratories were asked to print an accompanying question form, view the on-line images, answer the questions and to fax their completed form to UK NEQAS(H). The anonymised data returned was then referred to the DM project team in Manchester for analysis.

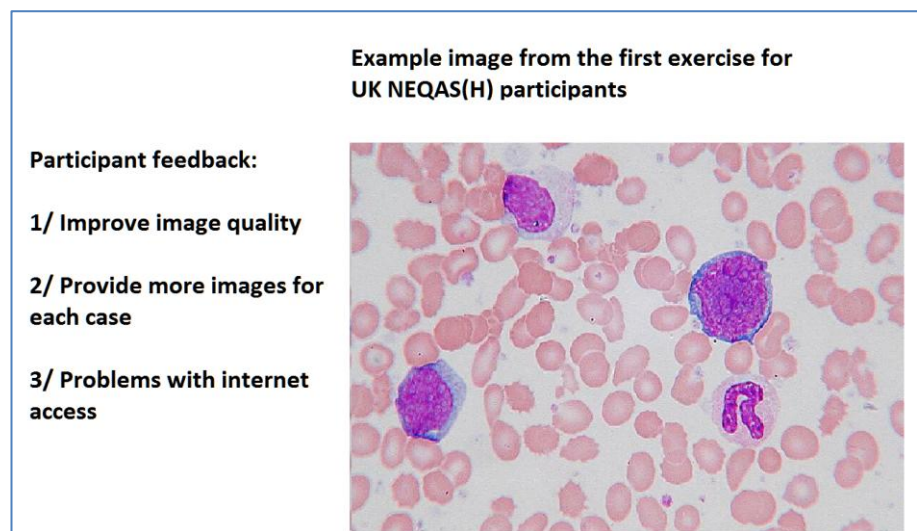


Figure 1.1 Example image from the first DM exercise for UK NEQAS(H) participants undertaken in 2000, as reported back at the symposium in October, 2001

Some 480 laboratories were sent details of this first DM exercise by UK NEQAS(H), of which 38 successfully completed the process (Brereton *et al.*, 2002). Considering the lack of sophistication of the computer technology available in some laboratories at that time (35% of responders completing reports said they did not have access to a PC at work, so had completed the exercise from home) the level of response was regarded as a success. Presenting the findings and feedback at the UK NEQAS(H) participants annual symposium “perspective in performance” in 2001, there was a sense of optimism and anticipation, from those who had participated, that the introduction of computer-based technology would bring innovation to the way in which laboratories could access verified professional material for education. Reviewing these initial exercises against the quality of modern images and current speed of computer downloads that early positive feedback now appears generous but it reflected the excitement and eagerness of an enthusiastic minority to embrace the digital revolution, just gaining momentum in laboratories. However, there was also strong criticism from some quarters at the symposium, who vehemently opposed the use of digital images and believed there would be no call to develop digital or internet-based techniques for laboratory morphology training. Concerns that the quality of images viewed over the internet would never be comparable with the actual view obtained via a microscope and that the entire DM process had no relevance to performing morphology evaluation by microscopy were eloquently expressed. Such concerns had genuine foundation, as poor quality images from unnamed sources

proliferated across the internet with little evidence of professional validation. It was clear to the DM project team from that first exercise that we would need to engage with the fears and expectations of concerned potential users if we were to succeed in developing a service which would be effective for all professionals.

1.7.2 Responding to participant requirements and changing technology

Each year a DM exercise was prepared in order for UK NEQAS(H) participants to drive the direction of the project, with feedback at the annual symposia. The question the DM team asked of itself was in two parts:

1. Could digital images of blood films be prepared and presented at a sufficient quality for them to be acceptable as a professional resource?
2. If digital images of high quality were made available how would UK NEQAS(H) participants want to use them?

In 2003, feedback from the exercises led the project team to concentrate on DM images for educational purposes as this was the route favoured by participants. There were still too many vehement objections to the use of digital images in performance assessment to support consideration of that line of development.

The team released 12 images with a series of individually numbered cells, an answer form could be printed, participants had to identify the numbered cells and return the completed form to UK NEQAS(H). The case selected was complex so that there were numerous features that could be found in any one single field of view. One hundred and twenty eight laboratories participated (30% of invited laboratories), a marked increase on the first exercise and indicative of the increase in internet availability in laboratories and an increased interest in using the internet as a medium. For the first time laboratories across the UK could debate a consensus approach to morphology. An example image is shown in Figure 1.2 below.

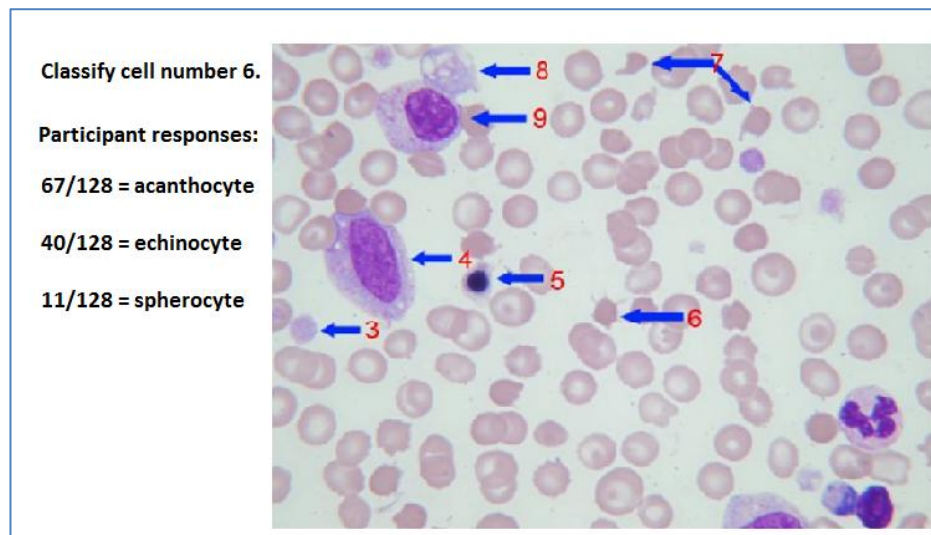


Figure 1.2 Example of feedback to UK NEQAS(H) participants following a DM exercise in 2003. The responses are shown for morphology feature number 6; the expected response from the options given was an acanthocyte.

The feedback reports showed there were clearly issues for users over the quality of the technology; of those participating 26% could not access the viewing program QuickTime™ from their place of work due to institution security policies and ‘firewalls’. Even though the images had been prepared using professional quality objectives and checked by the DM team, the quality of the viewing facilities available to users varied (Wells *et al.*, 2004). So there could be morphological debate by professionals *e.g.* for cell number six as shown in Figure 1.2 there are elongated spikes jutting out from the surface of the cell membrane, so laboratories could debate whether the erythrocyte shown was an acanthocyte or an echinocyte. Interestingly, however, eleven laboratories termed it a spherocyte, the project team did not know whether those 11 participants genuinely believed it was a spherocyte or whether their computer screen did not have the quality to allow them to see the contour variation of the cells membrane.

Along with debate over individual cells the team sought participants’ feedback on the educational element, in the form of annotation to certain images as shown in Figure 1.3

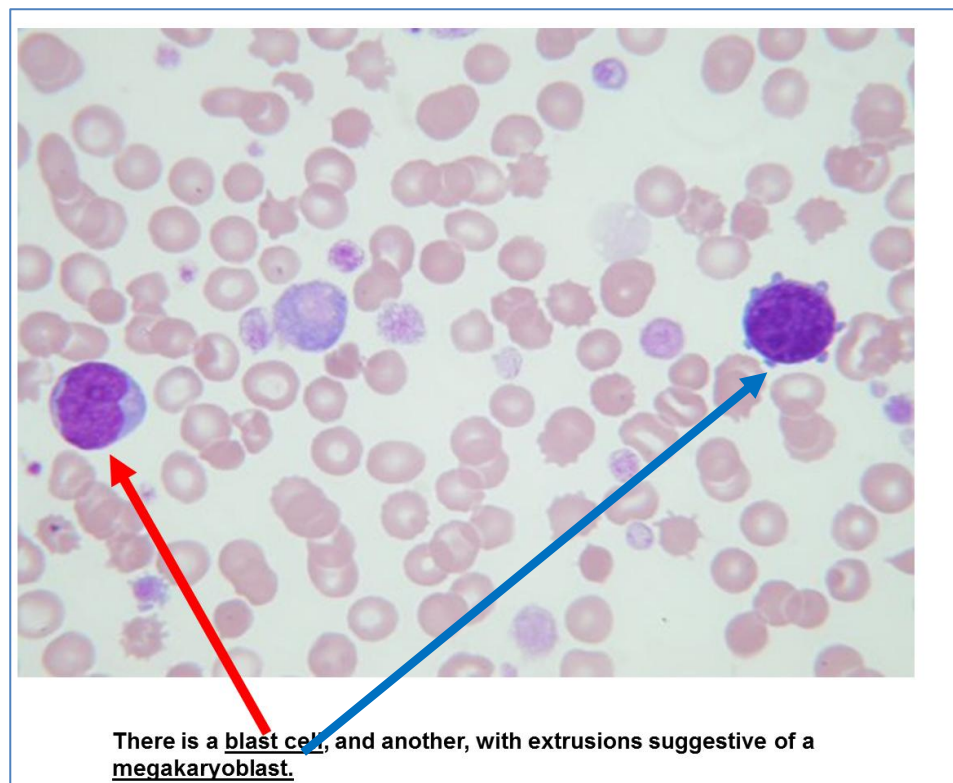


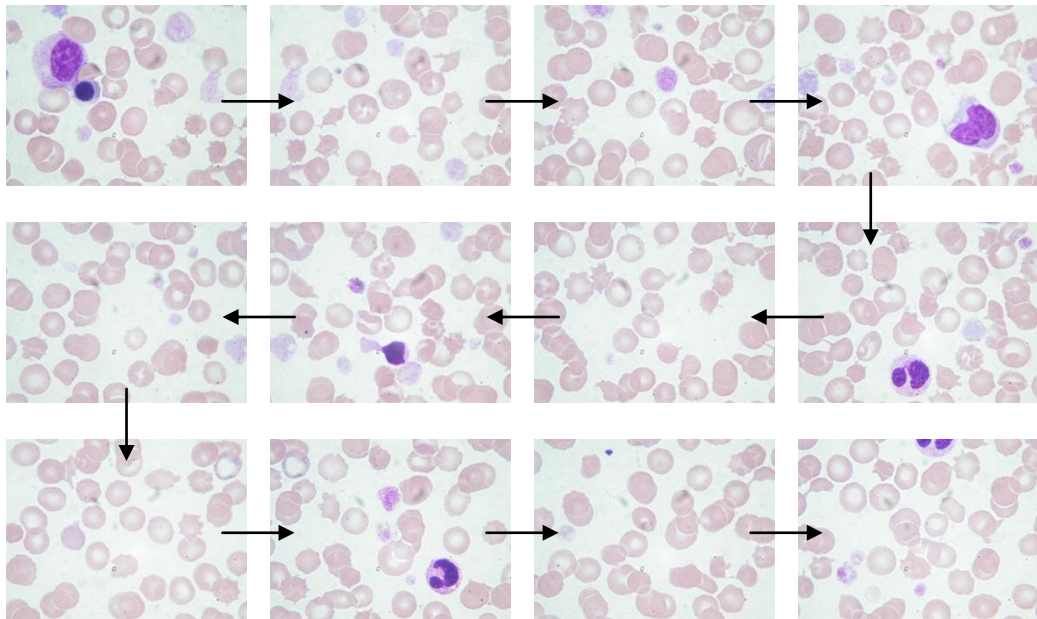
Figure 1.3 Morphological features identified for participants. Image originally presented without annotation or arrows, participants used a click of a computer mouse to display the arrows after first viewing the image.

1.7.3 Assembling images to provide a larger field of view

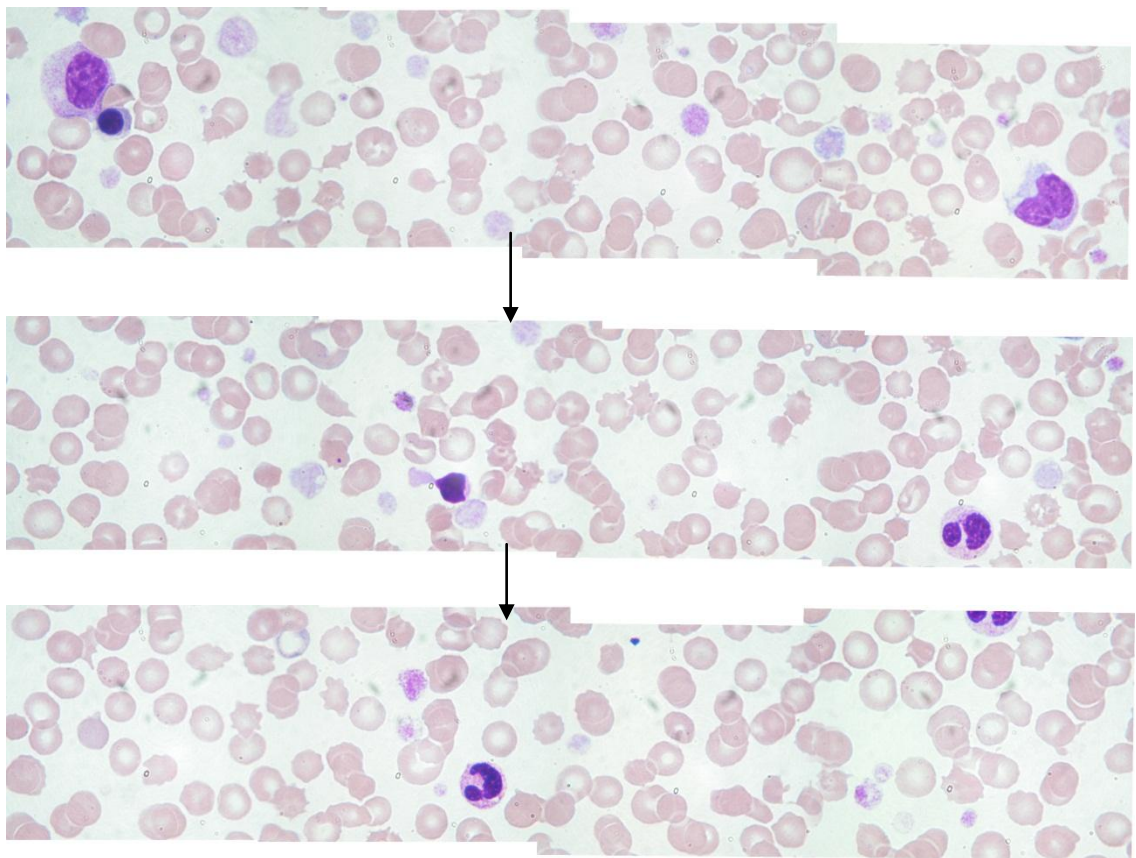
One problem previously identified with using single frame images, was that in order to obtain the required magnification to allow the user to “zoom” in on key features, such as the granularity of neutrophils, the original image must be taken using a microscope objective which is a minimum of x50 magnification. This meant that the overall numbers of cells that could be captured in each single frame was relatively few, whilst this was ideal for imaging single white cells, such as a blast cell, it was inadequate for creating a wide enough field of view associated with actual microscopy. If a lower power objective was used, *i.e.* x40 magnification, the size of the field of view was greater with more cells per field, but the definition of the final image not sufficient for examining fine details such as granules.

Image-processing software was being used in other areas to create larger blended images of good quality (Weinstein *et al.*, 2002). The next development had to be to increase the field of view whilst retaining the higher resolution. PanaVue™ Image

Assembler software was used to blend images that had been taken sequentially; this process was termed “stitching”. Initially 4 individual but sequential images were taken of a blood film, then blended together using the software. These mini-stitched images were then re-presented to the software as single images and the rows blended or stitched together as shown in Figure 1.4 panels A to C below. Participants were then asked to use QuickTimeTM software, available freely on the internet, which incorporated a “zoom” facility to view the final stitched image (Figure 1.4 panel D). Whilst innovative at the time (Bromilow *et al.*, 2003) compared to current technology those final images now appear of poor quality particularly for red cell morphology.

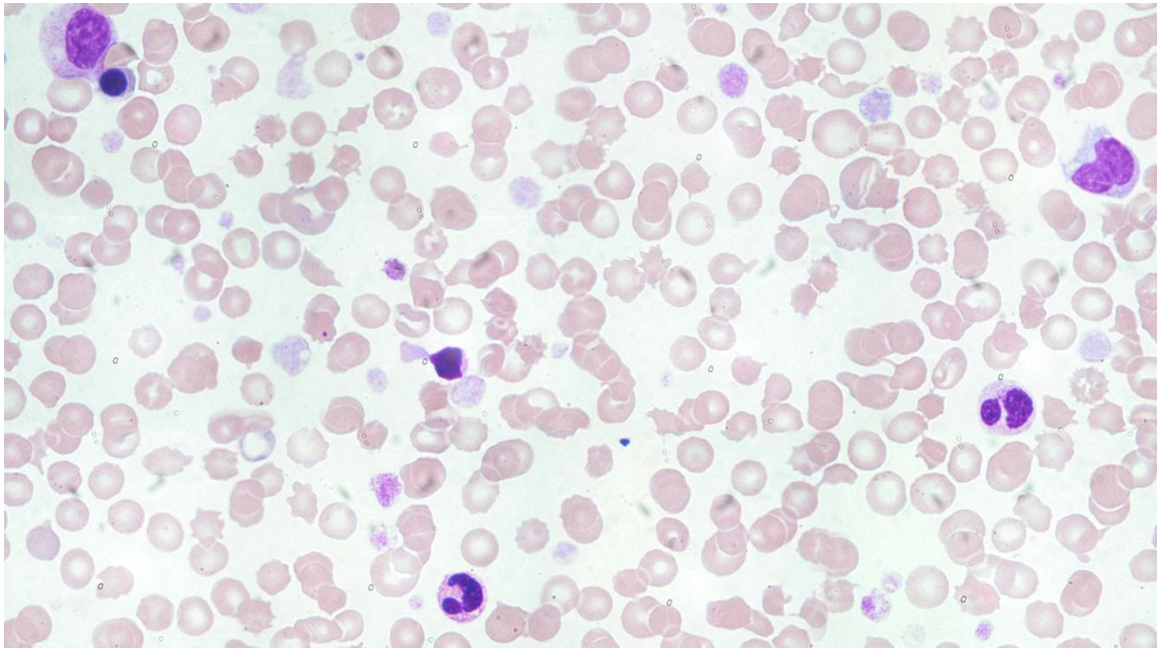


Panel A: Creating 3 rows of 4 sequential images

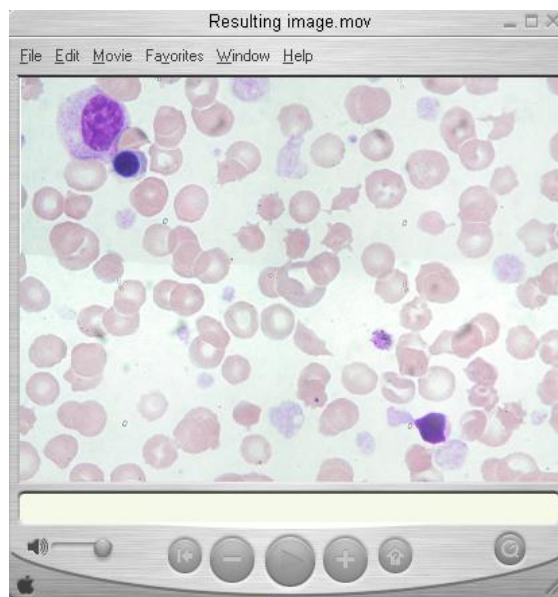


Panel B: Stitching 3 rows together using the PanaVue™ software

Figure 1.4 Creating a stitched image. Panel A: three rows of 4 sequential images loaded into PanaVue™ software for stitching. Panel B: three rows to be stitched together.



Panel C: The three rows of images stitched into one single image.



Panel D: Using QuickTime Movie™ to present the final image

Figure 1.4 Creating a stitched image. Panel C: final stitched image made from three rows of 4 single images. Panel D: stitched image viewed by QuickTime™ viewer to allow “zoom”.

1.8 Generating high quality images for digital morphology: technical challenges

The most commonly used format for image capture was the Joint Photographic Experts Group (JPEG). Images captured in other formats were often not found to be compatible with freely available viewing or storage systems, so images of high quality, but which require special software for viewing, or excessive memory for storage, are unlikely to succeed with potential participants.

The requirement, by haematology morphologists, to detect and interpret details seen within single blood cells is not generally required in histopathology where a dry x40 objective is employed for imaging (Furness, 2007). The level of resolution required must allow the haematology morphologist to define and separate the cellular elements, enabling them to see, for example, the granules of a neutrophil as definite and distinct entities and not a vague blur of purple colour. Individual granules less than one micrometer (μm) in size must be distinguishable from each other. This higher resolution demanded to view the features of individual cells and their inclusions (*i.e.* granularity or nucleoli), proved more difficult to achieve by automated systems. The use of oil-immersion with the higher magnification objectives (x50 and above) presented a particular problem for automated systems, as the process of physical movement of the slide beneath the objective, needed to create a large image, affects the refractive index of the oil, ideally manual refocusing of the cells is required every time the slide moves. This combination of an automated stage but with additional care taken to manually focus when required was not an attractive option to the developers of automated systems as it slowed the process, however the DM team believed this required more exploration.

The demand for high resolution of cytological detail creates a requirement for images to be of a higher quality than might first be expected. When viewing a blood film via a microscope the level of resolution is controlled by a set level of objectives, such that the highest resolution is achieved by a x100 objective, whereas the zoom facility on viewing software enable the users to “zoom in” to a much higher magnification. With no enforced zoom limits the viewer of an image on a computer screen expects the image to be clear at high zoom.

In the laboratory, blood films are routinely examined using an oil-immersion objective of x50 magnification or greater in order to examine the appearance of cellular inclusions which are only 0.2-0.5 μm in diameter. Such inclusions are resolved at the limit of the microscopes optical system, requiring the brain to interpret them as separate entities (Figure 1.5). For images to provide the resolution quality that professionals would deem suitable to train morphologists the images must be prepared with care to allow the examination of cellular structures such as granules (Hutchinson, Brereton & Burthem, 2005).

When using a microscope, the morphologist can alter the focus to gain a three dimensional view which the brain then translates, along with the visual information gained on the colour, contrast and texture of the cells to create a mental vision of a complete cell. The digital camera uses a “charge-coupled device” (CCD chip) which detects and focuses light via a series of units termed photosites, arranged together in sequence; these photosites each provide one electronic element, termed a “pixel”, of the final image. The CCD reads the accumulated charge at each photosite and an analog-to-digital converter changes the pixels charge into a digital reading in binary form.

A small secondary granule in the cytoplasm of a neutrophil might only be 0.2 μm in diameter but when magnified by the x50 objective lens it will be detected by the CCD chip as an area of 10 μm . This requires the chip to use between two and four adjoining light sensing units to capture the granule in its entirety. The chip then presents a final image made up of between two to 20 pixels. This enables the user to see the granules clearly in the final image; however, the viewing technology provides only a two-dimensional final view whereas the microscopist uses fine focus control to allow a three-dimensional effect.

The ability of the brain to create a textural view of the cellular inclusions is lessened when looking at images because the colour produced in every pixel is averaged across the pixel; this has the effect of further reducing textural contrast between features, most notably at the edges of features *e.g.* membranes of cells or their inclusions bodies. Furthermore each photosite only registers the total light it is exposed to, so red, green and blue filters are used (the Bayer filter pattern) with an alternating

pattern across the pixels to create mosaic of colour. Algorithms are then employed to average the colours across a pixel, taking into account the colours of the surrounding pixels, to blend the mosaic so that the greater the number of pixels, the more detail can be captured and the finer the resolution of the final image (Hutchinson, Brereton & Burthem, 2005).

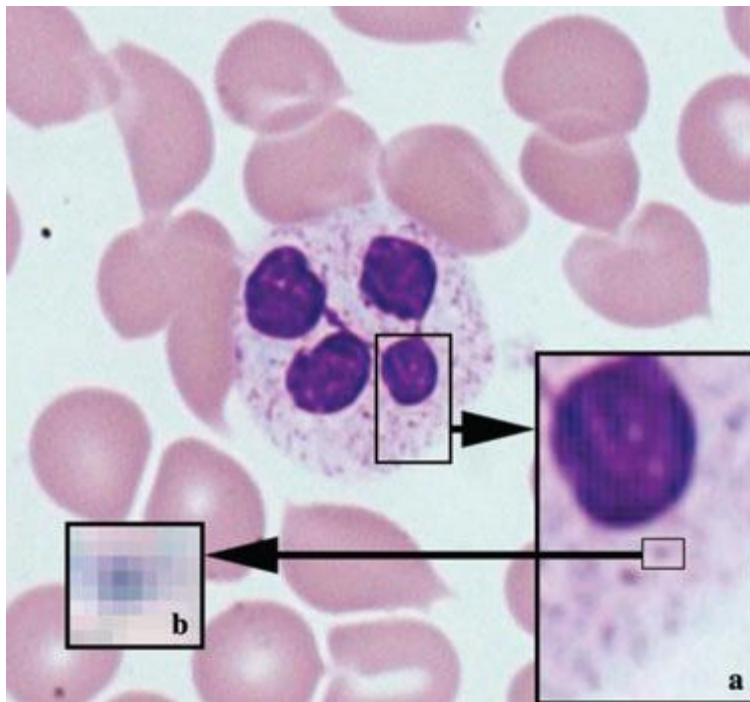


Figure 1.5 Digital image of a neutrophil showing typical granulation taken using a x100 objective lens. The detail of the image is shown in the inset panels. Panel (a) shows a magnified image of an intermediate sized granule, magnified again in panel (b) to show how it is constructed from individual picture-forming elements (pixels). In this case the granule is constructed from 12 to 16 separate pixels. The figure illustrates how the colour and contrast is ‘averaged’ in each individual pixel resulting in an indistinct outline for the illustrated granule (Hutchinson, Brereton & Burthem, 2005).

Initially cameras fitted to microscopes, including the Nikon Coolpix™ used in the early exercises for the DM project, were based on the technology found in the standard cameras used for general photography. This meant that the photosites were not evenly distributed across the camera but were found in greater density at the centre of the lens as suited to people taking recreational pictures, where the person or subject matter at the centre was likely to be of more interest than the subject matter at the

edge of view. The problem when using the Coolpix™ attached to a microscope being that the cells situated at the edges of the image were captured at a lower resolution than those in the centre. This proved particularly problematic when blending sequential single images together to create a larger image.

1.8.1 Developing large images taken at high resolution

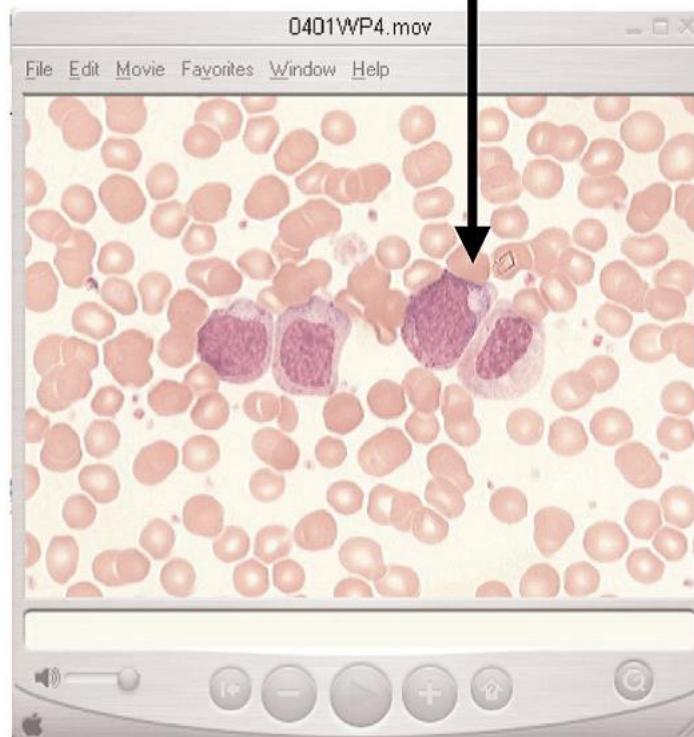
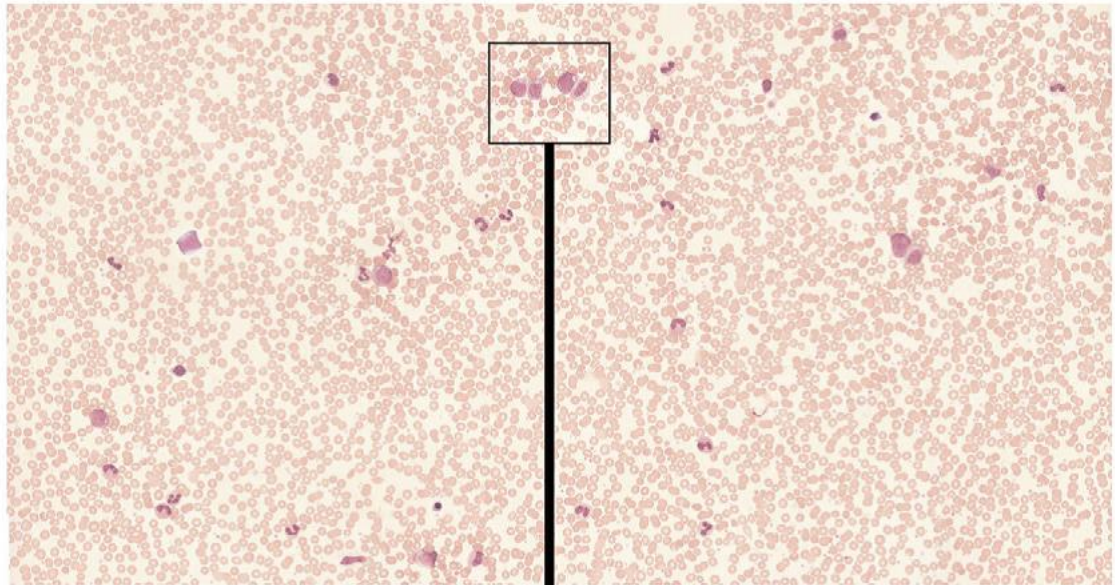
The response to the initial DM exercises had been positive with an increasing number of laboratories responding each year despite a lack of external publicity for the exercises. UK NEQAS(H) gave their participants the opportunity to engage with the annual exercises and the DM team gave feedback at the following annual symposium. Participation in EQA schemes is a well-established mechanism for improving quality standards and more than 500 laboratories were already registered with UK NEQAS(H) for their blood and bone marrow morphology scheme using glass slides. There was, however, no comparable morphology scheme available for those same professionals to participate in as individuals, so the DM project team selected this as its area for development.

In 2004 the DM team updated to a Nikon DN100 digital camera, designed specifically for use in the haematology morphology setting, attached to a Nikon Eclipse 600 microscope with a plan Apo x60/1.40 oil-immersion objective lens. The camera produced a colour image of a resolution of 1.3 mega pixels at a speed of 15 frames per second. With the photosite units distributed evenly across the camera the quality of resolution for cells at the edge of the image was equal to that of those cells in the centre. By manually adjusting the shutter speed and the aperture gain (Speed 1/500, A Gain) for each image the light could be better controlled to ensure the finer detail of cells was adequately captured (Brereton *et al.*, 2005). The network facility allowed the creation of images using file transfer protocol (FTP format) which could be easily communicated to users across standard network access. Importantly the system had software to allow the user to adjust exposure and sensitivity with adjustment of light and colour intensity across red, green and blue settings (interpolation). The quality of the actual Romanowsky stain used for the blood films varied in intensity even when performed with a controlled and standardised method. By their very nature different clinical conditions also impact on the final stain *i.e.* hypochromic red cells appear paler

than well haemoglobinised red cells and paraproteinaemia can increase the level of purple background stain on the slide. To a microscopist these are an acceptable and accepted part of blood film examination, but when taking images they add a layer of complexity in capturing the image. It becomes essential to perform a “white balance” for each blood film before taking any images, this simple procedure requires finding a cell free area on the slide, taking the image out of focus, the software will then take the colour of the area as the background and adjust the light and colour to remove unnecessary background shading.

Improvements in technology allowed the upgrade from the PanaVue™ Image Assembler software to Adobe photoshop CS™ with photomerge software, a more sophisticated system for blending single images taken in a sequence with an overlapping area. The final image was composed of 40 separate but overlapping sequential images and made available for participants to view using the freely available software package QuickTime™ example Figure 1.6 (Brereton *et al.*, 2005).

Depiction of Virtual slide 0403BF1 (CGL) 2.04 MB JPEG



Virtual slide 0403 BF1 in Quick time player™

Figure 1.6 Sequential images taken at high resolution (x60 oil objective) to create a large image or “virtual” slide and the resulting ability to “zoom” to high magnification using QuickTime™.

This work showed that creating a large image that allows the viewer the facility to “zoom” in to view individual cells of interest at a high resolution, sufficient to distinguish the inclusion bodies of both red and white cells, requires that the original image be taken with an objective above that of the x40 available on automated scanning systems. To achieve the larger image a series of sequential images must be taken and then blended seamlessly together. This process is best achieved using a semi-manual technique as each frame may require fine adjustment to focus as the oil readjusts with each movement of field.

1.8.2 The role of images compared to glass slide blood films for morphology

Using these techniques the DM team asked participants of the UK NEQAS(H) conventional glass slide morphology scheme whether DM images stitched from 40 separate high resolution images were adequate for educational or reporting purposes. Four cases were selected for imaging that had been distributed in previous years to participants of the conventional glass slide scheme. The cases were re-coded and participants were not informed that these DM cases had been seen previously. Cases comprised: 0001BF2 sickle cell anaemia (HbSS), 0403BF1 chronic myeloid leukaemia (CGL), 0002BF1 B cell prolymphocytic leukaemia (B cell PLL) and 0403PA1 malaria (*Plasmodium ovale* (*P. ovale*)).

Once prepared the images were placed on the CMFT website and linked to UK NEQAS(H) so that participants could enter via the UK NEQAS(H) website. The cases were selected to cover a range of red cell and white cell morphological features, snapshots are shown in Figure 1.7 below (Brereton *et al.*, 2005).

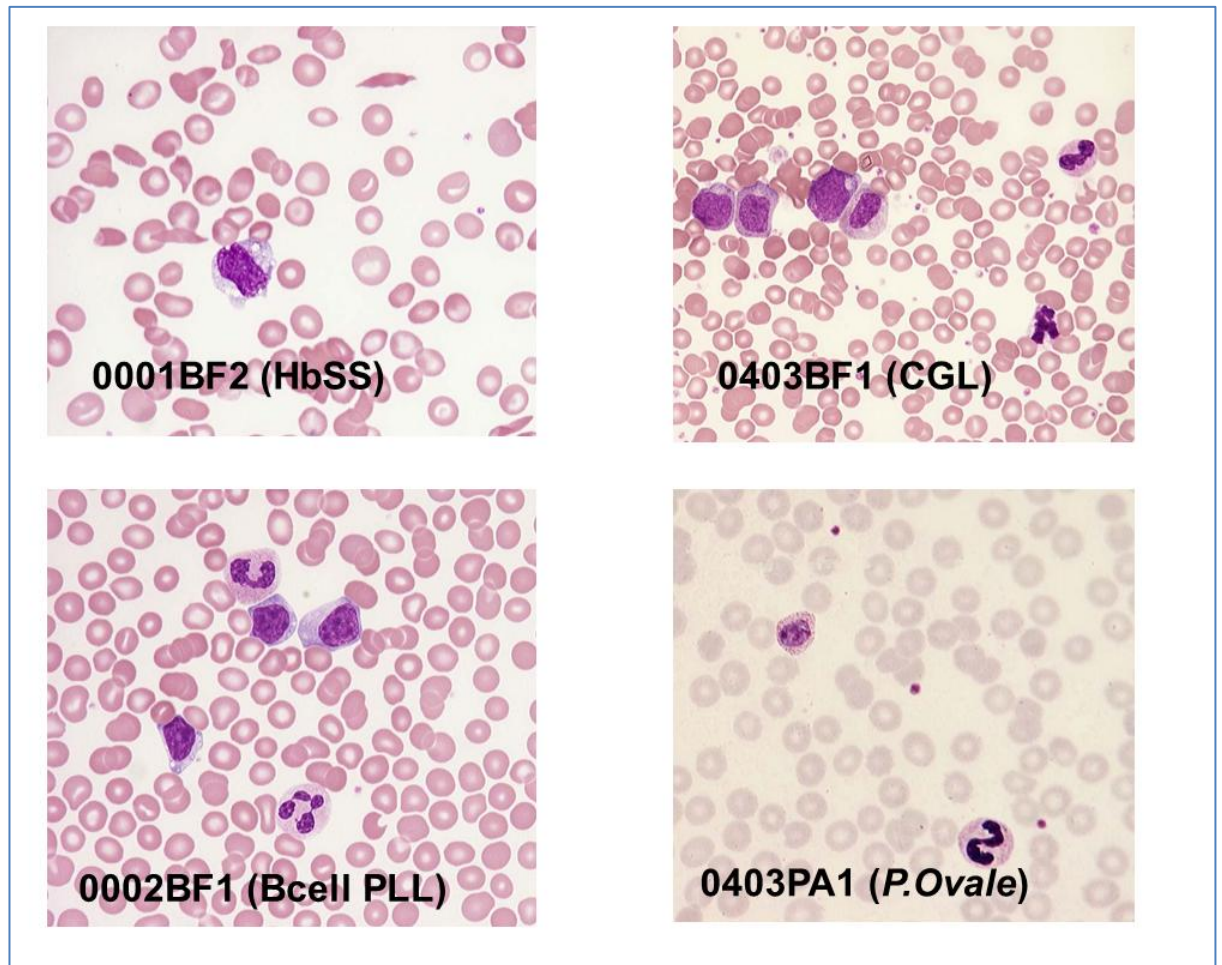


Figure 1.7 Example images from the 2004 UK NEQAS(H) DM exercise as reported back at the annual symposium. The images shown each depict approximately four high power fields stitched into a single image and showing key morphology features. The actual images used had a total of 40 fields each. The case number shown as given to the original glass slide survey (two were distributed in 2000 and two distributed 2004).

Encouragingly 166 laboratories participated (approximately one third of UK NEQAS(H) registered laboratories), viewing the images via the internet using QuickTime™. Participants were asked to select the top five most significant features exactly as they would for a conventional survey, and to answer a series of questions in order to rate their viewing experience (1 being poor to 5 being excellent). The responses for the morphological features were compared with the original returns from the conventional glass slide survey in which 400 laboratories had participated. Despite the images being comprised of only 40 stitched single fields participant morphology comments agreed remarkably well with the findings from the conventional surveys (Figure 1.8).

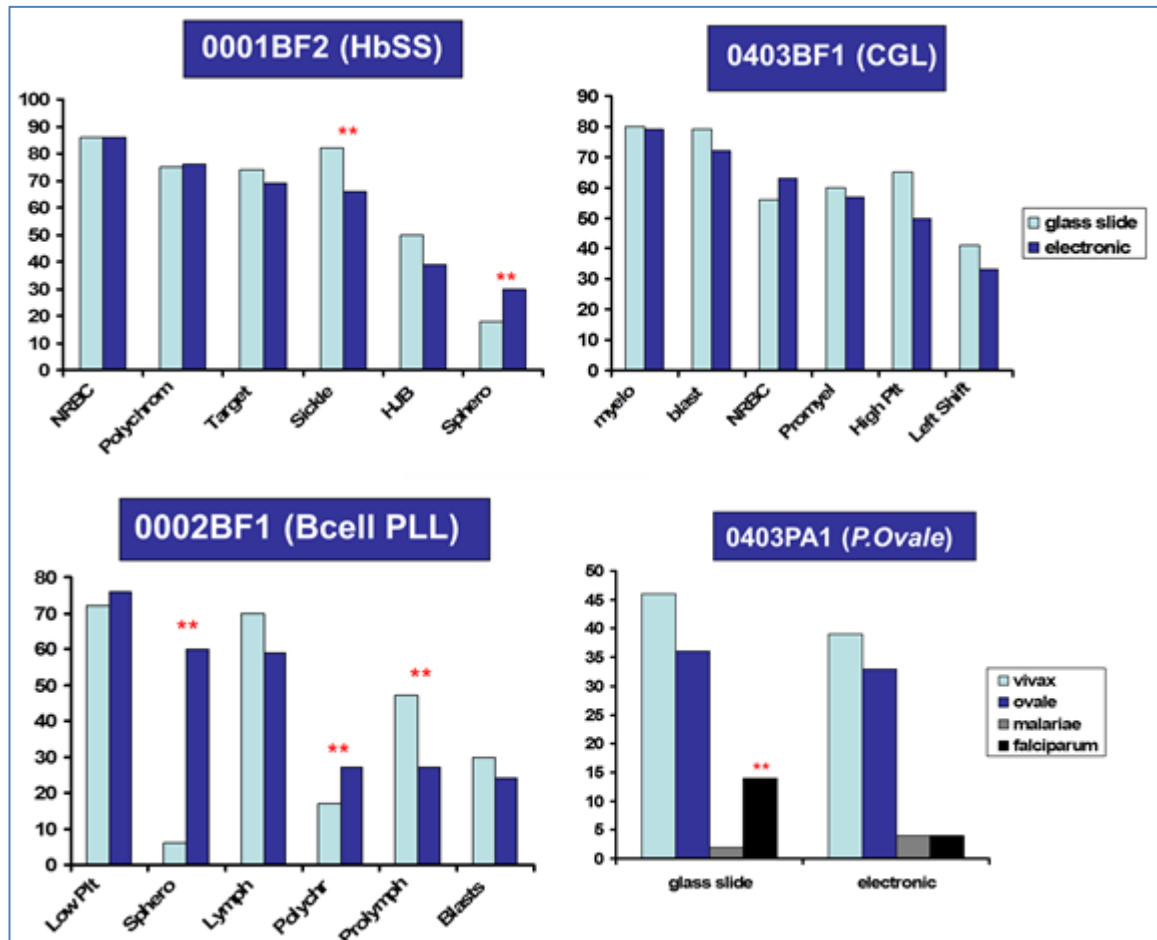


Figure 1.8. Comparison of morphological features selected from cases as reported from the conventional glass slide scheme and from the DM exercise. Features are shown as percentage selected by total number of those laboratories who participated (400 for glass slide and 166 for DM). The malaria chart from case 0403PA1 shows the species of malaria selected by participants of the glass slide and the DM exercise. Significant differences $p = <0.05$ are shown ** above the respective feature column. (Burthem *et al.*, 2005).

Of the top 22 morphology features selected across the four cases only three were significantly different, using Chi-square tests, from those found in the original survey (Figure 1.8). In the case of B cell PLL, spherocytes and polychromasia were reported by 64% and 36% respectively of centres evaluating the images, but these features had registered at lower levels (6% and 17%, $p < 0.05$ Chi-square test) when the glass smears had been distributed. Most likely these differences were due to the selection of the fields of view presented in the images, in review the collaborative DM team felt

that the area of smear depicted for the B cell PLL may have been from an area of the film where the red cells were spread too thinly affecting their appearance and accentuating the spherocytic elements of the red cells. This bias of the photographer was a concern and supported the need for image selection to be monitored with images validated by other skilled individuals. To add rigour to the process formal agreement that images be viewed by at least two members of the morphology SAG in addition to the two members of the DM team who had prepared them, was agreed by UK NEQAS(H) at the annual morphology SAG 2005.

In the image showing malarial parasites of species *P. ovale* the overall pattern of species selection was the same however fewer participants incorrectly identified the species as *P. falciparum* compared with the results from the original glass slide survey (4% compared to 14%, $p < 0.05$ Chi-square test). Again the problem of presenting only 40 fields of view was considered by the DM team as creating a bias *i.e.* with such a relatively small total area to view we were presenting the key features rather than letting the participant search to find them. When asked if participants thought the area of view offered for each case was adequate for morphological comment on average 76% responded positively however this dropped to 52% for the malaria case ($p < 1.01$ Chi square test), total numbers are shown in Figure 1.9. Despite this lack of confidence in image size, the results for the malaria case reflected the findings from the conventional glass slide survey, with the majority selecting the incorrect species of *P. vivax* (Figure 1.8). These findings suggested that the image correctly reflected the parasite morphology from the glass slide, despite the concern over the bias of field selection and of size of image (Burthem *et al.*, 2005).

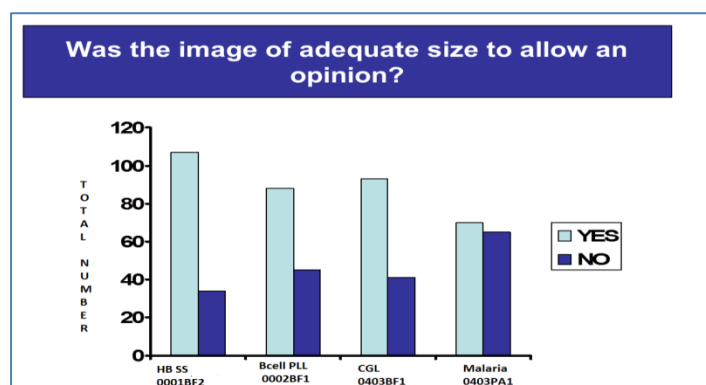


Figure 1.9 Participant opinion as to whether the 40 field mages were of adequate size for reporting purposes. Opinions of participants (n = 162) are shown at total for 4 morphology cases where YES = participant agreed image size adequate for morphological and NO = participant disagreed that image size was adequate for morphological reporting. (Burthem *et al.*, 2005).

When asked about the potential use of such images and the role UK NEQAS(H) could play in delivering them participant responses (n = 162) about their experience were mainly positive, with the use of images for teaching, and providing rare cases (bone marrow or paediatric cases) seen by > 90% participants as possible uses for the system. Furthermore 71% of participants stated that following the exercise digital images would be considered a useful addition to morphology quality assurance (details shown in Figure 1.10).

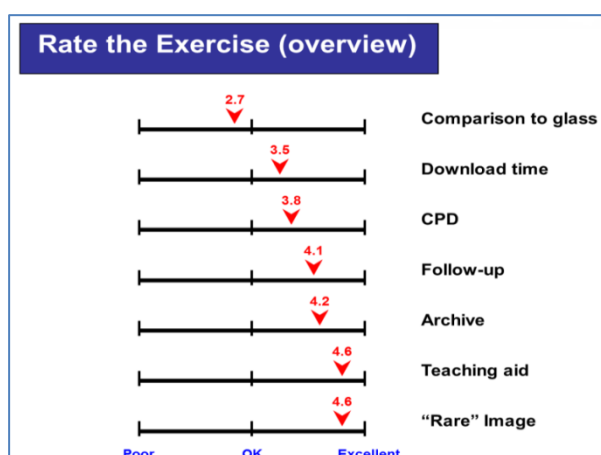


Figure 1.10 Participant responses to subjective questions following completion of the DM exercise. Results of 162 laboratories, shown as a mean of all participant responses, answers were rated 1 to 5 where 1 = poor or not acceptable, 3 = acceptable or agree, 5 = excellent or desirable. Participants were asked to rate their experience of examining the digital slides comparing them to conventional microscopy and considering the computer based experience and the opportunities and pitfalls (Burthem *et al.*, 2005).

1.9 Creation of the UK NEQAS(H) digital morphology library

Whilst continuing to test and improve the quality of “stitched” images the DM project team also considered how images should be presented to potential uses.

A digital image library of morphology cases was created for UK NEQAS(H) by imaging all glass slide blood film surveys distributed from mid-2002 (cases 0205BF) up to 2010 (Nambale *et al.*, 2010). The library was designed to be an internet-based reference package for participants. A formal process was agreed with the UK NEQAS(H) morphology SAG to ensure the library was of high quality and validated fit for purpose:

- Between 30 and 50 high quality single field images were taken from each glass slide case (a selection of both x60 and x100 oil immersion objectives were used).
- Images were reviewed for quality and morphological content by two morphologists at CMFT (members of the DM project team, usually myself and one other), reducing the number of images per case to between 15 and 25.
- These images were then copied to a compact disc and distributed, by post, to members of the morphology SAG for further review. A copy of the glass slide survey results was included to aid the review of morphology content.
- A final selection of between 8 and 16 images was then authorised for use by Dr John Parker-Williams (Consultant Haematologist providing expert comment for the UK NEQAS(H) glass slide scheme and member of the morphology SAG).
- The images were then presented alongside the FBC results and a minimal patient data set (age, sex) as used for the original glass slide surveys.
- Each case was presented on a different “web page” linked from CMFT to UK NEQAS(H) by a specified internet address. Users could enlarge each image to full screen by clicking on it (examples Figure 1.11).
- Some cases had extra links to other relevant data *e.g.* immunological markers, biochemistry results or cytogenetic information.

UK NEQAS

NHS
Manchester Royal Infirmary

NEQAS Survey Library.

Welcome to the digital image library of previous blood morphology NEQAS(H) surveys. You will find multiple images under each case depicting the morphological features reported in the original glass slide survey along side the clinical details and expert opinion of the case.

Surveys 2002
Case 0205: [BF1](#) [BF2](#)
Case 0206: [BF1](#) [BF2](#)

Surveys 2003
Case 0301: [BF1](#) [BF2](#)
Case 0302: [BF1](#) [BF2](#)
Case 0303: [BF1](#) [BF2](#)
Case 0304: [BF1](#) [BF2](#)
Case 0305: [BF1](#) [BF2](#)
Case 0306: [BF1](#) [BF2](#)

Surveys 2004
Case 0401: [BF1](#) [BF2](#)
Case 0403: [BF1](#) [BF2](#)
Case 0404: [BF1](#) [BF2](#)
Case 0405: [BF1](#) [BF2](#)
Case 0406: [BF1](#) [BF2](#)

Surveys 2005
Case 0501: [BF1](#) [BF2](#)
Case 0502: [BF1](#) [BF2](#)
Case 0503: [BF1](#) [BF2](#)
Case 0504: [BF1](#) [BF2](#)

Examples of Stitched Surveys/Images (instructions for use)
Stitched images have been created from multiple single images to give a larger field of view allowing movement around the image with zoom.
Note: To view these images, you will need the apple quicktime. After installation is complete, please refresh your browser.
[Case 0205bf1](#) - Stitched with feedback
[Case 0301bf1](#) - Stitched with feedback

0301 BF1

The five most commonly reported features were:
Nucleated red cells
Blast cells
Macrocytic platelets
Myelocytes
Megakaryocyte fragments.

Patient Details

Age (yrs)	55
Sex	f
Hb(g/l)	8.7
WBC($\times 10^9/l$)	76.7
Platelets ($\times 10^9/l$)	114

Dr J Parker-Williams, Scheme Director, UK NEQAS

prepared from a blood specimen taken from a fifty-five-year-old lady, who was diagnosed with AML-M7 in 1992.

with complex morphological abnormalities - too many to score as many stated! Myelofibrosis in 1992; a splenectomy was performed in 1996 for increasing splenomegaly; twenty percent recorded features of hyposplenism. The majority felt that the features were those of a myelodysplastic disorder with transformation to AML-M7.

and megakaryoblasts were present, many of the latter being micromegakaryoblasts; on typing 50% of the blasts were CD61 positive. Many large and abnormal megakaryocyte fragments were present. Thirty-five percent of participants declined to offer a diagnosis.

[Go to No 2 : Morphology Tutorial](#)

Figure 1.11 Screen shot from the digital image morphology library prepared for UK NEQAS(H). From a list of available cases one could be selected, leading to an overview of single shot images depicting the main morphological features alongside summary information. Each single image could be enlarged for view.

The DM library created a valuable resource for UK NEQAS(H) containing over 80 different morphology cases comprising more than 800 separate images.

1.10 The UK NEQAS(H) digital morphology pilot scheme: a two year trial

In order to test the theory that digital images would be accepted by laboratory-based professionals as an educational tool for developing their morphology skills it was proposed that the DM library be employed to create a UK NEQAS(H) pilot scheme. A series of cases was identified, with questions that participants could access via the

internet. The morphology SAG agreed a 2 year pilot scheme to be formally registered by UK NEQAS(H) with the IBMS in order for participants to use as evidence for CPD.

The pilot scheme ran from April 2005 (Harcourt *et al.*, 2006) closing September 2007, a presentation was fed back to participants at the annual symposium (Brereton *et al.*, 2008a) and a full report published (Brereton *et al.*, 2008b). In total 16 morphology cases, selected from the DM library, were issued in the format of 2 cases per release to individuals who registered for the pilot scheme with UK NEQAS(H). Registrants had to be professionals who worked in a laboratory which already participated in the conventional glass slide morphology scheme and the pilot DM scheme was limited by UK NEQAS(H) to a maximum of 500 participants. This limit was set in order to test the operational processes that needed to be put in place at UK NEQAS(H) headquarters and also to avoid overwhelming the project development team as no similar process had ever been attempted. UK NEQAS(H) had invited participating laboratories of the glass slide scheme to nominate one individual per laboratory to register and the first case went live to 221 individuals increasing to 416 individual registrants by April 2006. The majority of registrants were based in the UK (86%), however individuals also registered from 14 countries (Table 1.1). This was the first time UK NEQAS(H) had been able to distribute morphology cases to such geographically disparate laboratories and their participation confirmed that there was potential for an internet-based morphology product (Brereton *et al.*, 2007a).

Table 1.1 Location of registrants completing cases in the DM pilot scheme

Location of registrants	Number of registrants
UK	342
Eire	43
Portugal	12
Israel	4
Australia	2
Saudi Arabia	2
Sierra Leone	2
Falkland Islands	1
Gibraltar	1
Holland	1
Kenya	1
Oman	1
Spain	1
Sweden	1
Switzerland	1
United Arab Emirates	1

Registrants received email notification of each case release. The cases were selected from the DM image library, so all had been released previously as glass slide surveys but the cases were renamed. Case information was made available on separate “web pages” giving expert opinion, additional clinical comment and technical test results along with consensus data from the original glass slide surveys (Figure 1.12). Cases were scheduled for quarterly release and registrants were given four weeks to complete a questionnaire, selecting what they believed to be the most significant features morphologically and to complete a reflective feedback form. Within two weeks of the completion date forms had to be posted or faxed back to UK NEQAS(H) who then issued the IBMS CPD participation certificates to registrants by post. There was no process for electronic return of participant data or UK NEQAS(H) reports.

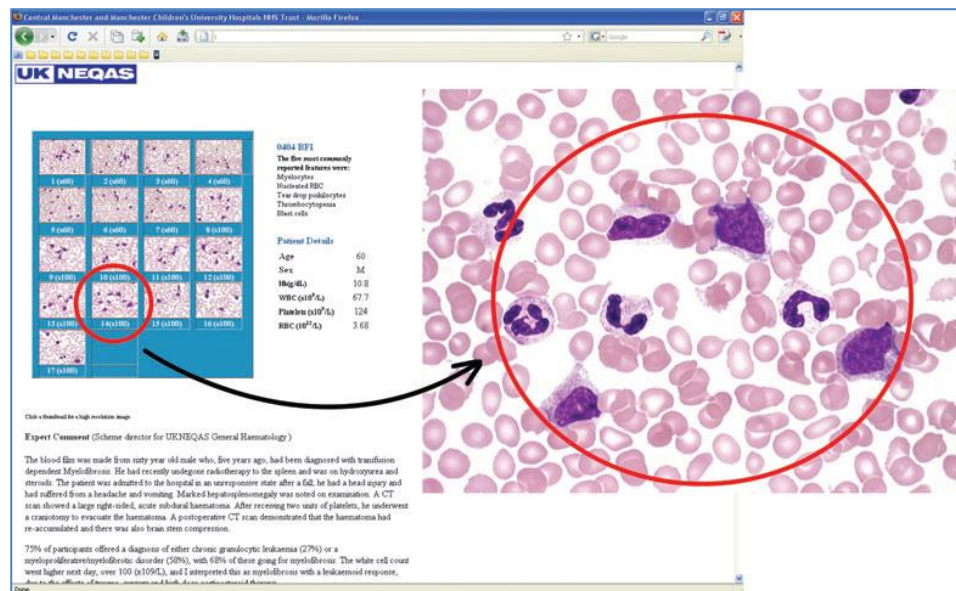


Figure 1.12 Screen shot of a library case from the pilot scheme. This case has 17 separate images alongside basic FBC data and a case history. Registrants could enlarge each image by clicking the PC mouse. A link at the base of the page took the registrant to the relevant documents they needed to print and complete. (Brereton *et al.*, 2008b).

1.10.1 Lessons learnt from the UK NEQAS(H) DM pilot scheme

Overall more than 50% of registrants fully completed the cases (lowest completion rate 19%, highest completion rate 69%) but there were substantial issues reported with internet access at some institutions. Feedback showed registrants had spent an average of 30 minutes completing a case but had also spent additional time on background reading around the clinical condition confirming that the total time spent on a case was about 1 hour which was considered acceptable for CPD purposes (Brereton *et al.*, 2008b). The free format reflective feedback, however, varied from just a few comments to several pages which proved difficult and time consuming for the experts at UK NEQAS(H) headquarters to assess. If a national DM scheme was to be developed with UK NEQAS(H) then serious consideration of how to get data reports to and from potential participants was needed.

Registrants' feedback stated that the benefits of completing cases were their increased awareness of both clinical and technical issues. More than 70% of those who completed feedback stated that involvement in the pilot scheme had improved their

knowledge of the clinical aspects of the morphology, with less than 20% saying their knowledge had not changed (improved knowledge varied with complexity of the clinical condition depicted). Comments were particularly positive from those in smaller laboratories where access to certain clinical conditions was unusual and feedback showed that registrants clearly felt the system had value for teaching and education in laboratories where time or expertise were limited. Whilst the quality of the images was rated highly by registrants, the criticism of the system mainly related to the problem that sets of single frame images do not provide a comparable experience to microscopy. (Brereton *et al.*, 2008b). When using a microscope the user can move across a blood film and assimilate information on the morphological appearance of the cells as they appear on a slide.

Despite all the problems with IT security, variable internet access, the operational problems of faxing and collating paper-based returns and the deliberations over the number of single images required, the UK NEQAS(H) pilot scheme had successfully maintained a completion rate of more than 45% over the 2 year period. Acknowledging the significant level of maintained interest by registrants the pilot scheme had also demonstrated that it was possible to produce high quality images that would be found acceptable to professionals as a validated form of education. The lessons learnt could be taken forward with the goal of incorporating high resolution stitched images (Brereton *et al.*, 2009).

1.11 Perceptions of image quality: workshop

As image quality was a key element of the DM project, the collaboration proposed a morphology workshop for interested professionals; UK NEQAS(H) scheme director, Professor Keith Hyde, invited participants and prominent professionals working in morphology. Both medical and scientific staff came to discuss emerging digital imaging technologies and their possible roles in EQA. The DM team were aware that digital images of blood cells, accessed from a variety of web sites, were criticised for poor rendition of stain colour, professionals deemed them not of the same staining quality that could be found in their own laboratories. However, as each laboratory sets its own internal standards and although the variation in staining times or stain concentrations may be small, the overall difference in the final appearance may be substantial. For the

UK NEQAS(H) glass slide scheme all slides distributed are stained at UK NEQAS(H) headquarters to their own standardised method, yet the returns show that up to 5% of laboratories report the staining to be unsatisfactory.

As discussed in section 1.4 it is likely that some of this dissatisfaction is merely that the stain used is different from the laboratories own, so morphologists become accustomed to the finer detail and nuances of the morphology using a stain they are familiar with and may find fault with a different stain. For this reason it is unlikely that regardless of how carefully the DM team try to recreate the colours, shades and contrast of a blood film under the microscope they simply will never be able to achieve each laboratories perception of how the film should look.

Prior to the workshop those agreeing to attend were sent two fixed, unstained peripheral blood smears, one with aberrant red cell morphology (sickle cell anaemia), and one with abnormal white cell morphology (chronic myeloid leukaemia). They were asked to stain the slides in their own laboratory and return the slides to the DM team for imaging prior to the workshop (sets of slides from 22 institutions were returned for imaging). The resultant images were randomised and presented at the workshop, attendees were asked to compare the images and vote for the stain which they felt depicted the best morphology due to stain quality. As two images for both cases received >80% of the votes and 10 images received no votes at all, attendees found themselves voting for another laboratory's stain. (Brereton *et al.*, 2007b). Less than 10 people voted images from their own slide and many acknowledged that their staining protocols had been set for many years, rarely revalidated or critically reviewed. Examples of 9 images presented at the workshop are shown as a montage in Figure 1.13, panels A and B.

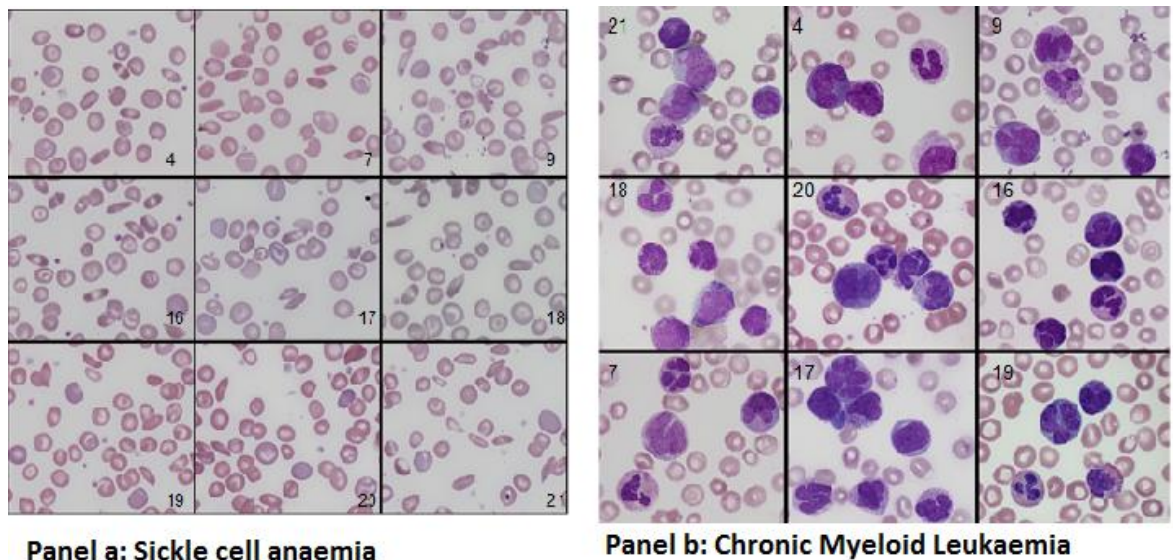


Figure 1.13 Examples of images taken from two cases stained by different laboratories. Images were taken sequentially in one session on identical microscope and camera settings so that no alterations in the imaging process occurred. **Panel A** highlights differences in the staining of red cells and **panel B** highlights differences in staining of white cells. (Brereton *et al.*, 2007b).

Although the number of participating laboratories was small (22), six different staining machines had been used and no two laboratories were found to use an identical staining protocol.

Once the quality of staining is assured, the capture of the true colour of the image must be considered if imaging is to best represent what the viewer can see down the microscope. The process of creating a digital image from a blood film adds further variation in colour and shading which needs to be considered and controlled at the time of image capture. The ability to change the colour saturation within an image could be used to improve a poorly stained blood film by manipulating the blue, red or green elements to intensify colour or change the hue. A lack of care at this stage of preparation, however, can detract from the quality of the final image by making the image unrealistic and reduce the satisfaction of the user. Figure 1.14 shows options that were available to alter the red, green and blue elements of two slides stained by two different laboratories.

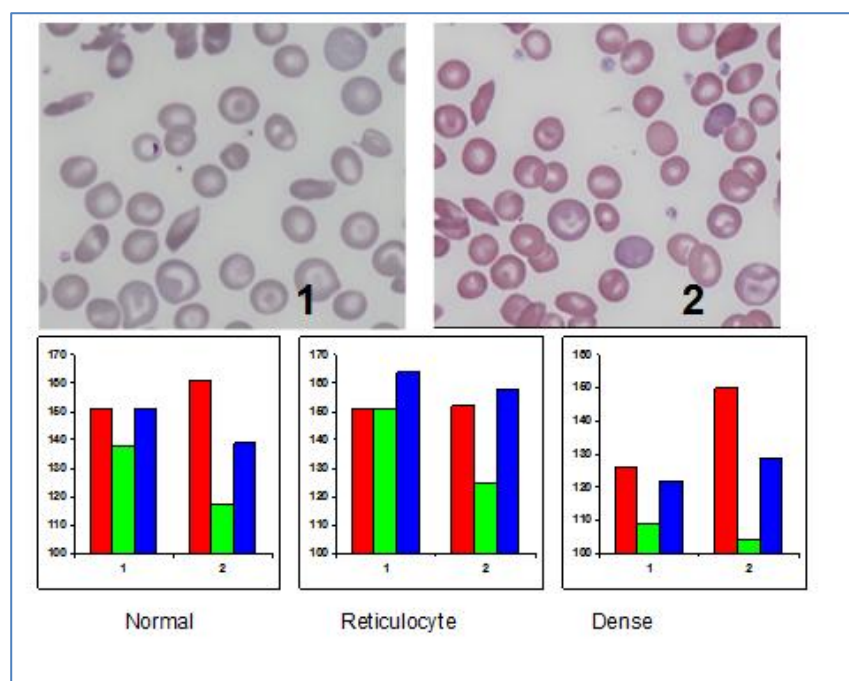


Figure 1.14 Images taken from the same case (sickle cell anaemia) but stained in different laboratories using different staining protocols. The bar charts show the different red cell colour saturation (normal red cells, reticulocytes or polychromatic cells and dense red cells without central pallor) due to the different laboratory staining protocols. The colour differences are shown as detected by the software (for red, green and blue) for images 1 and 2. (Brereton *et al.*, 2007b).

Once the image is captured the quality of the display must be capable of presenting the high resolution used to capture the image, with no further effect on colour, thus standards of display are also needed. Software enhancements at both the point of image capture and that of image manipulation could reduce, rather than enhance, user satisfaction if not considered and controlled. In 2006 UK NEQAS(H) were able to give instructions to participants for setting up their monitors for optimal viewing.

Beginning with the first exercise in 2000 the collaboration had worked with participant feedback to progress the digital imaging of blood cell morphology for professionals. The work summarized in this chapter culminated in the decision to develop a full national scheme using digital images of morphology cases for educational purposes to be part of the UK NEQAS(H) portfolio. The governance, successful launch and early analysis of the scheme are presented in chapter two.

Chapter Two: The UK NEQAS(H) digital morphology scheme for continuing professional development

The DM project team was conscious that there had to be sufficient capacity within the collaboration for a scheme to deal appropriately with participant expectations. The reputation of UK NEQAS(H) as a leading provider of EQA and with a proven record for delivery of quality services could not be put at risk. The collaboration needed to prove that the scheme could be robust, from a technical standpoint, and manageable by UK NEQAS(H) headquarters for prospective participants. Monthly telephone meetings were put alongside six monthly face to face meetings, with the image development and presentation format driven primarily from Manchester with the administration, security and data integrity being controlled at UK NEQAS(H) headquarters. As an entirely new development for UK NEQAS(H) a DM scheme for CPD could not be launched or operated without rigorous testing so additional help was sought from members of the morphology SAG who were asked to participate in testing.

2.1 Selecting equipment for the UK NEQAS(H) Digital Morphology project

Whilst the DM pilot scheme had been operating advances in DM technology for pathology microscopy had been significant, with all the major suppliers of haematology microscopes offering some form of imaging system. Feedback from UK NEQAS(H) participants completing the annual exercises had highlighted their increasing expectations for both quality of image and ease of access for delivery. Early enthusiasm by some registrants had lessened the trauma of slow download times and varied image quality, although if a national scheme using this technology was to be successful all of these problems needed to be overcome.

2.1.1 Selection of microscope and camera system

Advice was sought from support specialists and also from histopathology colleagues already using digital systems for teaching to ensure money would not be wasted on overly complicated systems which might outperform requirements. Microscope manufacturers have related software packages aimed at simplifying and optimising the

image capture process and include the ability to set white balance, colour correction facilities and image alteration capabilities (sharpness, fine focus, brightness). Standard image manipulation packages can also be used for viewing, selecting and refining image content including the movement of cells within the image.

Taking sequential, overlapping images at high power is time consuming and requires patience but by moving across a smear a strip of images can be collected which can then be blended or “stitched” together to produce a larger image. Using appropriate software to stitch several overlapping strips together a large section (comprising 40 to 100 fields) can be imaged providing a “virtual smear”, or at least taking the part of the smear which is representative of the whole.

Available imaging technology for haematology was reviewed and equipment trialled with the support of the companies involved, microscopes were with their associated cameras and recommended imaging software. Notably the number of pixels in a camera lens had increased from 1.3 megapixels per frame (DN100 used at that point) to between 5 and 12 megapixels. Companies were also asked to include their best quality objective lenses. The main microscope suppliers had automated stages in development, or already in their portfolio, so were asked to provide systems with and without this facility as the team remained unsure that a fully automated system could provide a consistent level of focus across the whole image and wanted to test this option.

The emergence of full slide scanning technology had proved of interest in Histopathology laboratories where the problems around long term storage of glass slides was well documented (Weinstein *et al.*, 2002). The market leader for slide scanning was AperioTM whose Scanscope OS technology used a fully automated system to create images of whole tissue sections at x40 dry objective (leicabiosystems.com, 2017). At three to five times the cost of a semi-automated microscope system the Scanscope OS was seen as expensive but it was at the forefront of slide scanning technology and so included in the equipment assessment by the DM project team.

Initial testing included:

1. The Leica DFC490 and PAXcam3 digital camera and motorised stage

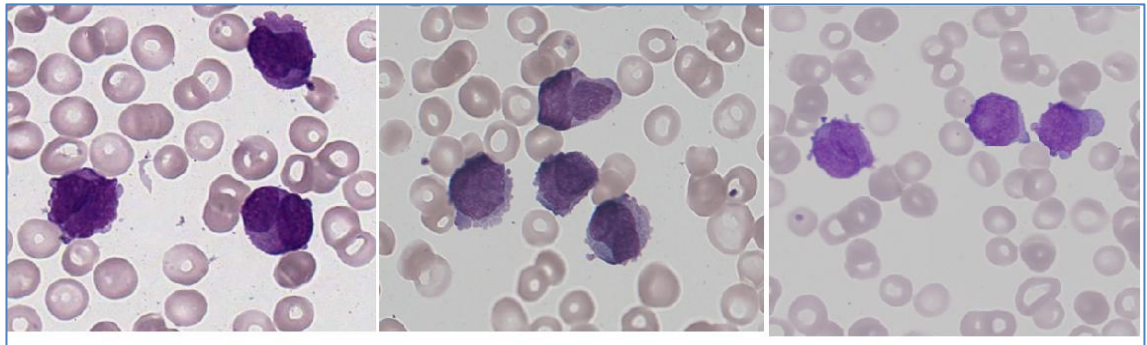
2. Nikon Eclipse microscope, with and without automated stage, and DXM1200F camera and DS-Fi2 camera (with IR-cut filter and enhanced frame rate). Nikon offered two different camera system so both were trialled.
3. The Aperio Scanscope OS automated slide scanner with x 40 dry objective.
4. The Zeiss M1 Axio microscope and imager with automated stage and Axio Cam HR Camera.
5. Olympus microscope with DSCF0025 “slide” camera system.

At that time the Leica and Olympus systems were not fully validated by the manufacturers so more extensive testing was continued with the Aperio, Nikon and Zeiss systems. Four cases from previous UK NEQAS(H) glass slide blood cell morphology scheme were selected for imaging by all systems. Cases had been selected to provide a range of white cell and red cell morphology features as depicted in Figure 2.1

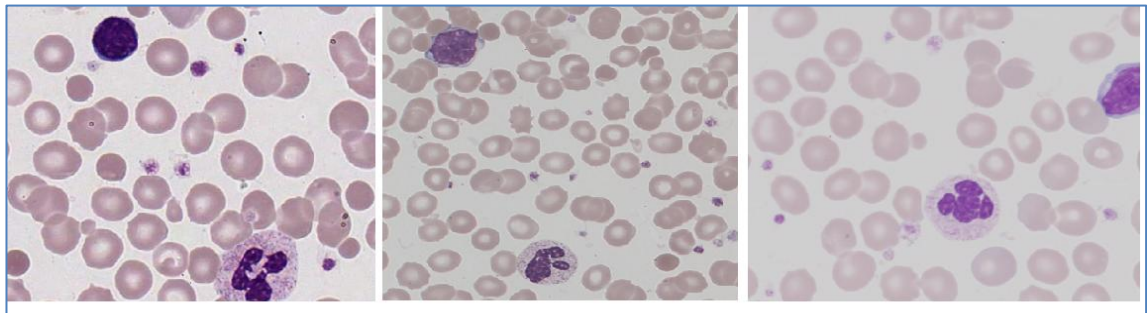
Panel A

Panel B

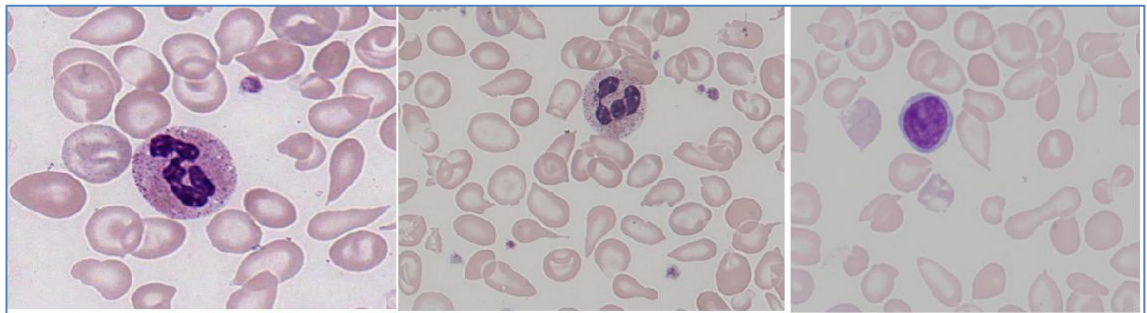
Panel C



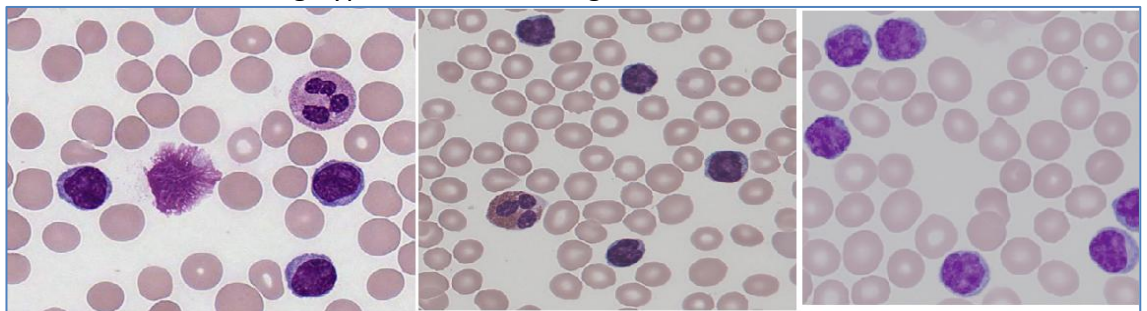
Case one: Acute promyelocytic leukaemia (APML): atypical promyelocytes



Case two: From a patient with severe burns: microspherocytes.



Case three: Haemoglobinopathy HbAE beta thalassaemia; multiple red cell abnormalities including hypochromia and target cells.



Case four: T cell prolymphocytic leukaemia: atypical lymphoid cells with nucleoli.

Figure 2.1 Snapshot comparative images from three different imaging systems used on four different cases. Panels A = Aperio Scanscope with x40 dry objective. Panels B = Nikon DS Fi L2 DC and eclipse microscope. Panels C = Zeiss Mi axio imaging system and microscope. All snapshots are prior to manipulation with colour correction software, variation in resolution was dependant on the magnification offered by the manufacturers on their systems. Both microscope systems offered plan apochromatic oil objective lenses at x60 or x63 and x100.

The systems were tested against a list of criteria namely; ease of use (settings, fine tuning of resolution, colour control), speed of process, size of final image and image file size, flexibility of software (ability to annotate images, ease of interaction), viewing software (ease of navigation of final image and download process) and quality of image (resolution and fine detail at zoom). The final images produced were viewed by four morphologists from the DM development team, each reporting independently on quality of the image. Consideration was given to availability of technical support and to overall costs. The findings were presented back to participants and at the British Society of Haematology congress (Sibanda *et al.*, 2009). The equipment that most met the criteria of the DM project was found to be the semi-automated Zeiss Axio Imager and this system was selected as the standard tool for UK NEQAS(H) DM projects and for the development of a national DM CPD scheme Figure 2.2

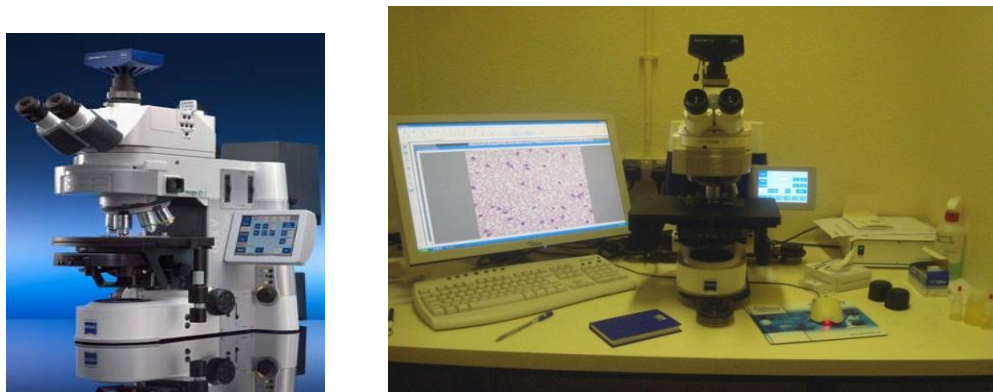


Figure 2.2: Photographs of the Zeiss Axio M1 imager system used for the creation of images for the UK NEQAS(H) DM project (Sibanda *et al.*, 2009).

2.2 Optimising the image

When examining a blood film the haematology microscopist rarely needs to look at the entire smear, usually the area being examined covers less than a third of the smear. An experienced morphologist will move quickly over a number of fields to gain an impression of the normality of the overall film whilst looking for the presence or absence of abnormal cells. In doing so they confirm that all cells seen are normal, both in number and ratio to each other, and that the overall morphology of features seen is consistent with the numeric full blood count data and any clinical details given (Bain, 2015). This is an intuitive and subjective process during which the morphologist aims

to provide information to the clinical team that will be additional to the numeric result data and can be interpreted in a clinical setting to impact directly on patient care.

Whilst single frame images provide excellent educational material on individual cells such as blast cells, they give a disjointed view of the relationship between different cells which is part of the microscopy experience. This is particularly important for assessing the level of abnormality of a blood film such as the frequency of damaged erythrocytes or the impact of platelet clumping on the numeric platelet count. The most widely accepted semi-automated blood film scanning system in the UK is CellaVision™ which concentrates on imaging separate white cells but provides a larger stitched image for red cell review (CellaVision, 2017b). Participants of the DM exercises and the DM pilot scheme constantly gave positive feedback about the quality of the single frame images for education purposes, however they wanted the facility to move across an image in a way that would better reflect their experiences of using a microscope. The challenge faced by the DM team was to build larger scale images that allowed the viewer to scan across, as they would do when viewing a blood film, without losing any quality of resolution required to look at cells in detail when necessary.

Unlike histopathology, where the architecture of the tissue sample and the relationship of cells within the structure are important diagnostic criteria, haematologists view blood cells which have been smeared on to a slide, so while the number and type of cells are diagnostic the spatial arrangement is not structured. The different approach required for imaging these preparations is that the histopathologist requires capture of that architecture, thus large scale images are required, whereas the morphologist in haematology only needs to examine an area of the preparation large enough to contain the diagnostic features. The problem is deciding how big an area is sufficient to capture those features. In blood films where the white cell count or platelet count are abnormally raised, or the diagnostic morphology markedly abnormal, a relatively small area of view, equivalent to a couple of fields, may contain all key diagnostic criteria. In a blood film, however, where the white cell number is reduced, or when abnormal cells only represent a small proportion of the total, a larger number of fields will need to be examined.

For images to be used for education purposes criteria are required to determine how large an image should be and what features it must contain. The advantage of not having to image the whole smear must then be balanced against ensuring that the final image is of sufficient magnification to capture essential cellular morphology. A disadvantage of large images may be increases in download time for the viewer, if moving across the image is slow or problematic the user will simply lose enthusiasm for the whole process.

For the UK NEQAS(H) DM project exercises all images had been checked for morphology content and image quality by at least two morphologists and all images were then submitted for critique and validation by at least two other members of the UK NEQAS(H) morphology SAG. This process was seen as essential by UK NEQAS(H) who, by definition, are an organisation with professional quality as their business. The previous work had also confirmed that, for haematology, the ability to focus on the finer morphological detail of cells with an image was deemed more important than attempting to replicate the entire smear or very large fields of view.

2.3 Demands for the virtual slide: stitching images

The DM team and morphology SAG had accepted that large images produced at low magnification do not provide the cellular detail required for haematological morphology. Images would need to be large enough for users to feel confident that they had covered sufficient cells to make a successful assessment of features and that 40 stitched fields was the lowest limit of acceptance. Using the Zeiss Axio Imager M1 microscope system with semi-automated stage, HRc camera and plan Apo x63 oil immersion objective lens at 1.4 magnification the team implemented a project to produce morphology cases made of between 50 and 120 individual sequential but stitched images. Each field would be checked and manual adjustments made to focus as required (Figure 2.3).

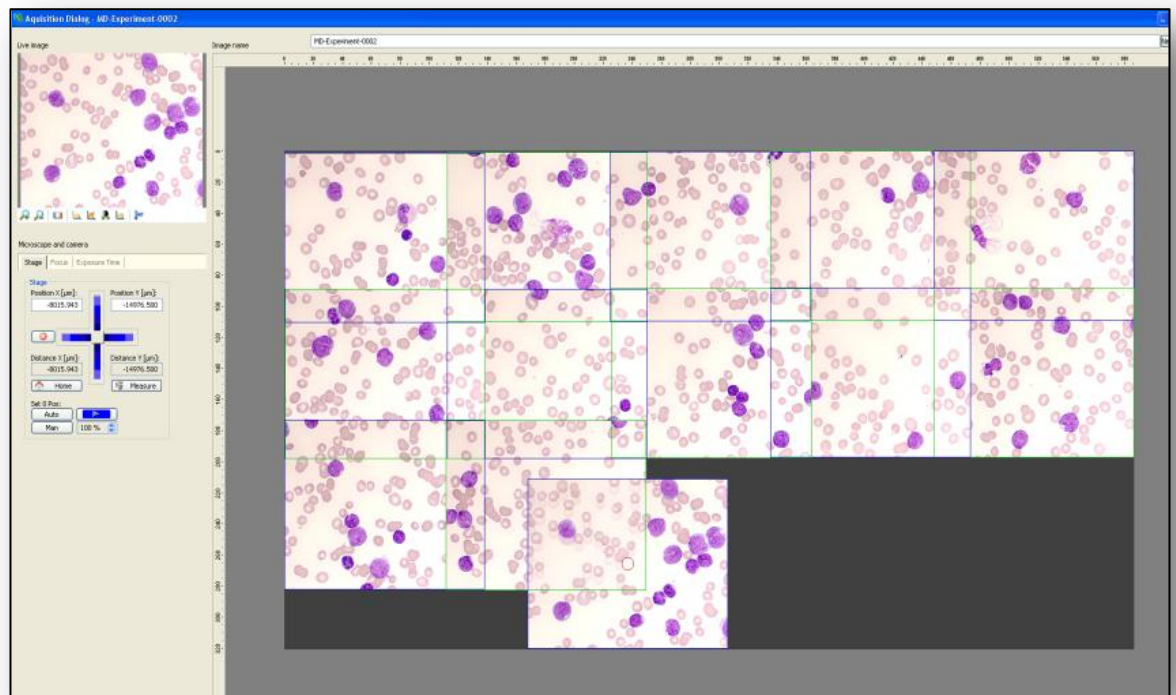


Figure 2.3 Creating a large stitched image from sequential high power fields. The single field view (top left of screen) can be magnified so that the operator can check and alter the fine focus to perfect the definition of an individual cell, and then click to take the image. By moving the stage manually to the next field of view, but watching the screen, the operator can ensure there is sufficient overlap between the fields for the sequential images to be stitched later on a PC using the adobe software. For the DM project at least six rows of nine images each would be required.

2.4 Expectations of the image from potential participants

A product aimed at delivering images for teaching and training in the modern healthcare environment must not only have a reliable software delivery system with established security guarantees but must deliver quality images (Goasguan *et al.*, 2006). No matter how sophisticated the software tools or how interactive the product, if the images are not of a high quality it will fail to deliver the service required by professionals and users will lose interest. Time is precious, both to managers and staff, so professionals must perceive that the selection of cases to examine and the quality of image meets their training requirements. If quality is low, interest not sustained, or benefit to the user not proven the product will fail.

Adobe Photoshop CSv5 photomerge function would be used to stitch the large scale images required by participants. Post-processing included adjustment to image brightness and contrast, colour balance (Curves function) and sharpness (Unsharp Mask) to ensure that reproduction matched the corresponding glass slide appearances, then images were uploaded to the viewing software (Digital SlideBox, Leica BiosystemsTM).

2.4.1 Manipulation of the image: adding and removing features

Careful consideration has to be given when selecting cases so that images meet the expectations of the user *i.e.* an experienced morphologist will have different needs to a trainee. The images must contain any and all necessary morphological features of the clinical condition they are attempting to depict. Moreover the features displayed must be in a realistic proportional relationship to each other as would be seen in a blood film. This may be difficult to achieve if image size is to remain manageable for the user and requires skilled manipulation of the image, it is essential that the image contains all the salient features needed to report the condition it represents, but that these do not appear unrealistic in their quantity or relationship.

Following taking the original sequential images for a case, extra single shots are taken from different areas of the blood film to capture any important features that might not have appeared in the area of film being captured for the stitched image. It is essential to take these extra single images at that time so that the camera settings and environmental conditions are the same. Going back to take extra images at a later date, even using the same settings, can produce mild but noticeable differences in the colour balance, meaning that when trying to add those features to a completed stitched image they will not blend correctly and look unrealistic. If taken correctly features may be added in to an image using Adobe Photoshop version 3, and subsequently versions 4 and 5, to ensure the final image contains the required elements. This process requires patience and should not be used indiscriminately; an example is given in the following Figures 2.4 and 2.5 panels A and B.

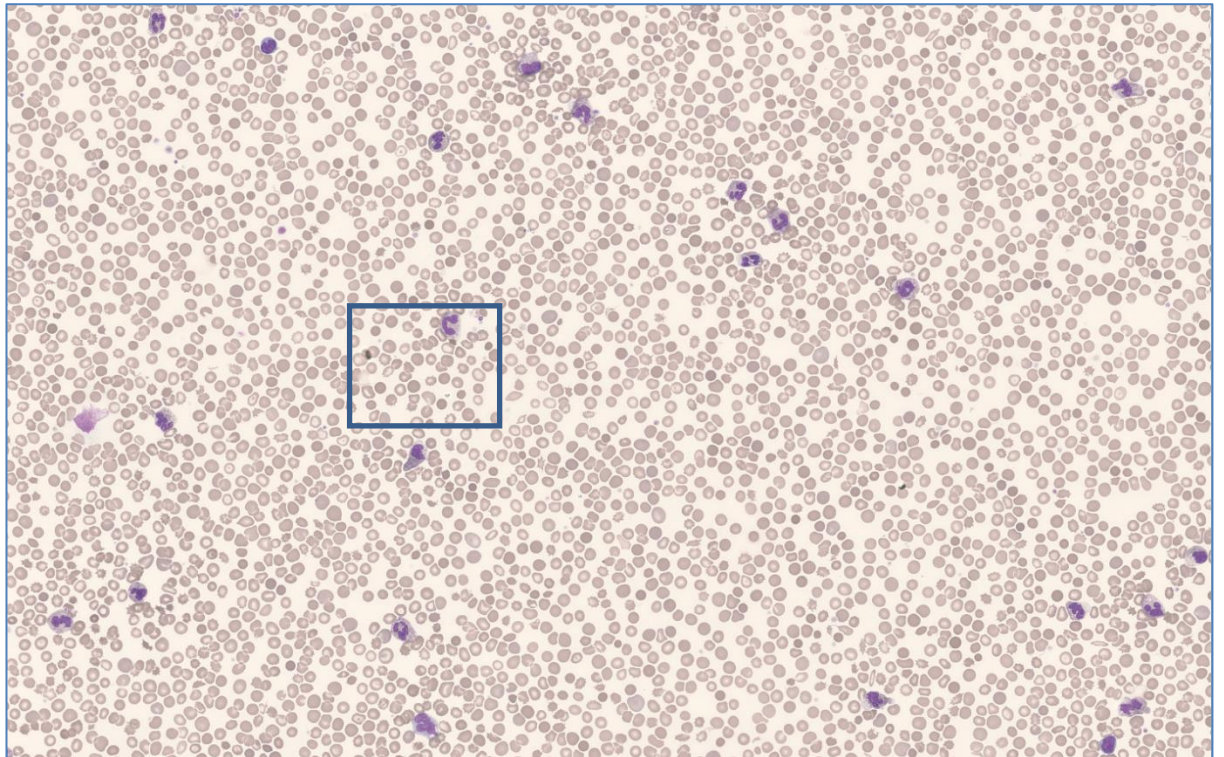
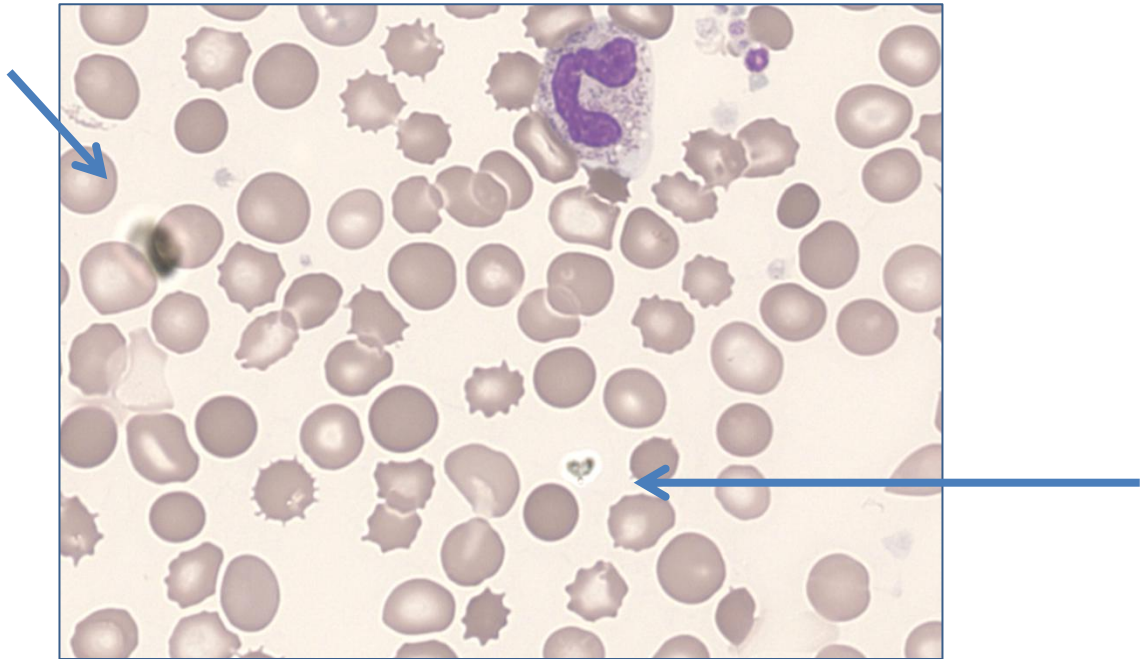
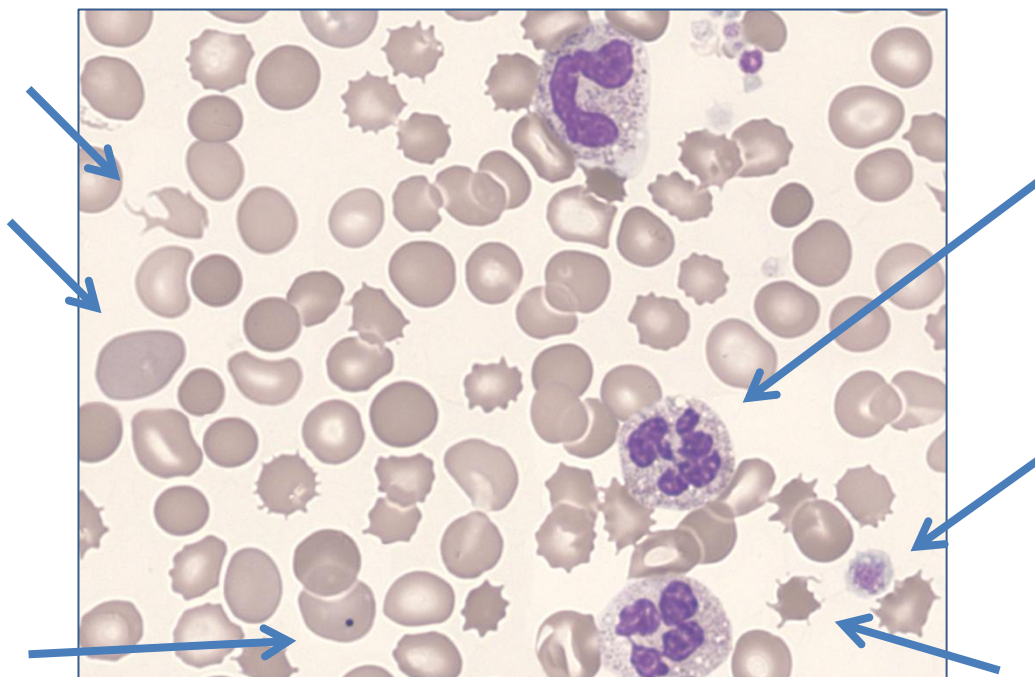


Figure 2.4 Large image created by stitching eight rows of eleven single sequential images. Following this process careful manipulation to remove faults in the image or add further features will be required. The area marked (black box) is selected for manipulation using Adobe Photoshop as shown below.



Panel A: Post stitching of image but prior to manipulation



Panel B: Image after manipulation

Figure 2.5 Magnified section of image pre manipulation (Panel A) and post manipulation (Panel B). Panel A shows dark reflective marks, probably dirt in the oil, which detract from the image quality and may confuse users. Panel B shows the faults removed and replaced with additional features typical of the case including red cell fragments, acanthocytes, a large platelet, a red cell containing a Howell-Jolly body, more neutrophils and large polychromatic red cell. These features reinforce the pathology shown; the Howell-Jolly body is a significant feature that was missing from the area captured originally but was present in the blood film.

The DM development team submitted this image preparation format to UK NEQAS(H) to be used for image generation for the proposed national DM scheme and the morphology SAG sanctioned this at the annual meeting 2007.

2.5 Criteria for case selection

Criteria for case selection required additional considerations specific to display in the digital image format that had already been agreed for use. A case must contain all the essential features of a condition, whilst the image must also appear natural so that the viewer can search for a rare feature even though only a representative area of limited size is being presented. The agreed image size was sufficient to allow the viewer to examine the morphology in realistic manner. As the whole slide would not be captured the morphological features representing the clinical condition from which the image is taken must be present. To achieve this some manual manipulation of the image may be carried out to ensure specific cells, not present in the fields selected, are added to the final view, as described in section 2.4.

The scheme would be aimed at all laboratory professionals who are either reporting blood films or training to do so. As the scheme would be for individuals, rather than institutions, the cases need to offer engagement with the participant whether that person is relatively new to morphology or whether they are an experienced reporter of blood films on a regular basis. The cases need to be of varied morphological complexity and have to offer challenges that appeal to the differing experience levels of the participants. The criteria for case selection required by the development team were:

- Is the case morphologically interesting? *i.e.* are there a variety of features to examine. For example cases which contain abnormalities in more than one cell type such as myeloproliferative disorders.
- Is there a clinical complexity to the case that will allow the inexperienced trainee to identify basic morphological features correctly but ensure an interpretive element to engage the more experienced morphologist? For example chronic lymphocytic leukaemia (CLL) with treatment associated haemolytic anaemia or a case of myelofibrosis in transformation to AML.

- The process of manipulating the image may include adding specific cells such as blast cells or Howell-Jolly bodies, required to achieve the correct outcome, but the image must appear to be as realistic as possible. Pasting in too many features or too many abnormal cells can negate the reason for selecting the case in the first instance and may lead participants to expect that concentration of abnormality in a real patient.
- The creator of the image is selecting the morphological features that the viewer will examine. It is essential the image creator understands exactly which features need to be present in the image to fully represent the case.

To satisfy these criteria early cases were chosen by the DM team and their selection and presentation agreed with the consultant lead, as the scheme became established possible cases were reviewed at the UK NEQAS(H) annual slide selection meeting, with experts from the morphology SAG agreeing the cases. Possible cases had to be of good quality staining as any flaws in the original film such as dirt, or artifact, would become more prominent when presented as an image on a computer screen. After preparation all images were checked by a member of the DM team and authorised by the Consultant lead prior to the case being built and then tested by at least two members of the team.

The first case selected for the DM scheme, beta thalassaemia intermedia post splenectomy (0801DM) had originally been used in the UK NEQAS glass slide scheme (0501BF2). This allowed UK NEQAS(H) to compare returns for the morphology features selected with previous consensus data from the glass slide scheme. Both schemes showed the same top five consensus morphology features, thus enhancing validation and provided reassurance to the morphology SAG that the process of image capture was fit for purpose.

In order to provide some feeling of familiarity for participants the presentation of data with the cases mirrored that of the conventional scheme in that brief clinical information was presented with the image alongside minimal FBC results (usually white cell count and haemoglobin concentration).

2.6 The requirement for secure and robust data management

All NHS Trusts and most independent laboratories have IT protection systems which can prevent users from downloading data from unauthorised internet addresses. The problems with access to QuickTime™ demanded a solution; navigation over the image gave participants a positive experience as they could move around the image and gain a more representative “feel” for the morphology. QuickTime™ was freely available but there were other limitations, the actual viewing of the image did not expand to cover the PC screen but was limited to a window covering less than half of the screen. During the DM pilot scheme the team had used the remaining space to present clinical data alongside the image but feedback had shown that this was not entirely satisfactory, users wanted the image to fill the screen.

The DM pilot scheme had used a series of single images to negate the problems by allowing simple viewing software to be used but the demand by participants for larger stitched image was a clear driver for change. Concurrently with the development of a suitable image, the DM team worked to resolve such problems and develop interactive viewing to allow users to examine images in a way which could mimic the movement of a microscope, without the need for downloading commercial software. To ensure all individuals could participate, the DM team looked at independent software companies with experience in EQA to host the images on a secure server and provide a secure data management system. In 2007 UK NEQAS(H) announced a contractual link with the software development company SlidePath™ and the team began development of a DM product for EQA use.

The introduction of an electronic proforma for morphological feature selection and the use of computer software to automatically create consensus data and provide participants with real-time feedback was seen as essential. The operational issues UK NEQAS(H) headquarters had experienced during the two year pilot scheme had left no doubt that to continue with paper feedback reports was not practical for large numbers of individual participants. Paper reports also introduced unnecessary delay; users require at least a portion of immediate feedback to gain a sense of achievement on submission and to retain interest.

Previous exercises, including the DM pilot scheme all involved paper returns as part of the feedback. If a digital scheme were to be introduced, and be open to all, then the entire process must be electronic, with data input by the participant immediately incorporated into a result bank and instantaneous production of a report presenting the participants responses alongside pre-entered expert data.

2.7 Challenges designing the question format

The challenge of setting questions relevant to the image needed addressing from two different aspects; firstly developing a case format that would be workable operationally by those building cases and also for participants interacting with cases, as well as enabling UK NEQAS(H) to organise reports. Secondly; ensuring that the content of the case and the workflow process provided a worthwhile and enjoyable experience for the participant, if not participation would be short lived.

2.7.1 Technical development of an electronic question format

Developments were worked through with potential participants at the UK NEQAS(H) annual participants symposia and demonstrations and exercises held at the IBMS congress morphology sessions (2009, 2011, 2013 and 2015). Feedback was then used to develop the format for questions along with Slidepath™ via monthly teleconference and bi-annual meetings.

Once users viewed an image they needed the question to appear alongside the image so they could check their responses against the image, with the ability to view either the image separately or the questions separately if they preferred. The structure of the questions needed to follow the same overall format for each case so that users would become comfortable with the format. Three main areas were agreed for the questions.

A. Question one. Format for selecting morphological features: As the majority of potential participants were expected to be BMS and, therefore, familiar with the successful UK NEQAS(H) glass slide scheme for blood cell morphology, it was decided to base the on-line morphological feature selection list upon that already in use for the glass slide scheme. Some alterations were required, for example more single abnormal cell options were required as the images were

of smaller area than a glass slide, and the parasite options were omitted for consideration at a later date.

Rather than recreate the table format already used by the glass slide scheme, it was decided to create a decision tree to encourage those reporting morphology to think about cell development in a stepwise process *i.e.* to consider the lineage, maturity, shape and granularity of cells rather than selecting from an alphabetic list. It was anticipated that participants would examine the image, decide what features they thought important to report and then open the tree by category of morphology feature.

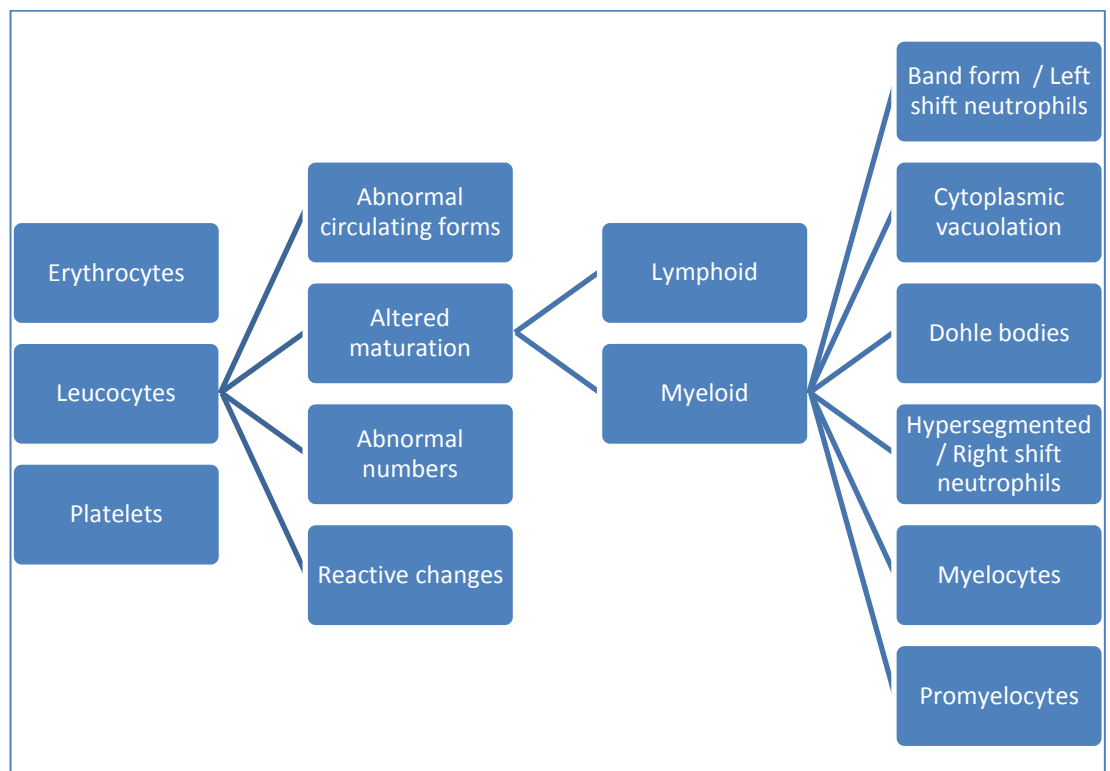


Figure 2.6 Decision Tree. Representing one line from the selection of leucocytes, to altered maturation, to myeloid, to the morphological features selectable.

There would be no limit, or minimum requirement, for the number of morphology features that could be chosen from the available 74 in the system but, after submitting their selections the system would demand participants gave a priority rank to their top five (1 being most important and 5 being least). This would encourage participants give consideration to their findings and only the ranked features would be used for consensus reporting.

B. Question two: multiple choice question (MCQ). The question would include up to five possible pre-set answers for the participant to select their one preferred or most appropriate option. This enabled the format to be designed once by SlidepathTM and enabled the DM team to set a different, appropriate question to suit each case. The person actively building the case needed only to type in their question and the 5 possible answers in to the template. The question could be about clinical or scientific interpretation *e.g.* “what clinical condition do you think most likely to be represented by the features you have chosen” with a series of options; or the question could be about subsequent action *e.g.* “From the features you have selected what further action might you take?”

Inevitably the MCQ was designed to ask the user to interpret their findings in order to reach a decision about what subsequent action they would take. This was a totally unexplored area of morphology reporting by BMS, yet interpreting the features they see is essential if they are to supply information to the clinical teams to fully support patient care, or to refer abnormal cases appropriately to medical colleagues for clinical interpretation.

C. Question three: free format summary. This final element of user interaction caused the most debate amongst the development team and morphology SAG prior to go-live and remains a challenge in the current scheme. It was agreed that, as in the glass slide scheme, participants should be encouraged to suggest a morphological diagnostic category for the image they had just examined. This would further encourage participants to consider the morphology features they had selected and the pathological processes involved and should be related to their actions taken in the MCQ.

The attraction of a free format text box to participants is that they feel no pressure to be precise and can be as broad with their interpretation as necessary. There are no constraints. In order to support CPD it was anticipated that participants might want to present their final reports in the workplace as proof of morphology education. They may, therefore, want to include extensive comments to demonstrate their understanding of a particular morphological condition. For the glass slide scheme, where laboratories are encouraged to

suggest a morphological diagnosis and where there are no penalty points for an incorrect entry, more than 50 % of laboratories complete this part. As the new scheme, however, would be aimed at individuals, the total number completing DM cases was expected to be much higher. This anticipated quantity of free text would be difficult for the expert reviewing the reports to filter into diagnostic groups and also for UK NEQAS(H) compiling the final reports. The more people enlist to the scheme the harder this is to cope with.

The alternative to a free format text box was a list of possible morphological diagnoses that could be selected from a drop down list, however, this proved more complicated than the initial thought might suggest. For a preselected list of diagnoses to have a true non-bias effect on the participant it would need to cover every possible diagnosis that might be received and every way that they might be expressed. Unlike diagnostic bone marrow reporting which may follow reporting guidelines (Arber *et al.*, 2016) blood films are examined on a myriad of conditions and various combinations of conditions that make generating an exhaustive list impossible. It would also be difficult to include complex cases where more than one pathological process was shown *e.g.* haemoglobinopathy SC with concurrent acute monoblastic leukaemia. If participants had to trawl through lists, even if they were alphabetical, this might prove frustrating and reduce user satisfaction. The team decided they had no choice but to allow free text for the scheme launch with a view to reviewing options at a later date, this problem has remained on the agenda for every morphology advisory group meeting since 2008.

2.7.2 Setting the questions: clinical and scientific relevance

To appeal to the widest range of laboratory-based professionals the format of the questions had to be inclusive to all levels of morphology expertise. If the questions demanded very specific responses, such as an exact diagnosis, those learning to report might be reluctant to express an opinion and so not submit. There also had to be an element of personal achievement, if the person got the correct morphology features or overall diagnostic group but not the specific diagnosis the scheme did not want to

put them off trying again for the next case. In the laboratory BMS refer difficult and clinically abnormal films to clinical colleagues, so should not be penalised for refraining from fully interpreting their findings in a quality assurance and educational setting.

2.8 Designing the feedback mechanism

From the early exercises (section 1.7.2) participants wanted educational feedback, this was seen as an important value added element for the new DM scheme and would need to be available once they had evaluated a case. The Slidepath™ system for this project had to allow images to be annotated and for hyperlinks to educational web sites to be included. When reviewing unattached hardcopy reports a user cannot guarantee that they are reviewing the same cells the expert opinion is referring to, but with an electronic narrative attached to an image the exact cells described can be located at the click of a button. For inexperienced morphologists, unsure as to the significance of the features described, related hyperlinks can transport them immediately to the relevant further information. The team worked with Slidepath™ to provide a customised system for both the educational narrative and the annotations to be linked at the touch of a button to elements within the system and also to external sources of educational material (these are shown diagrammatically in Figure 2.7).

2.8.1 Preparing the narration and annotations

The narration must be written with the image already prepared and accessible to the case builder on the Slidepath™ system, whilst not visible to other users, to ensure the narrative accurately reflects all the key features in the image. When reporting for the glass slide scheme the expert can make general comments about the overall morphology as everyone has a different slide to view, but when narrating an image of finite size the narrative has to be specific about the cells that everyone is going to examine.

There must be a logical flow to the narrative, initially referring to an overview of the general morphology at low power, then providing more detailed explanation covering the key elements of red cells, white cells and platelets both from a numeric and a morphological stance. The narrative and its conclusion must tie together the features seen without disclosing the definitive morphological diagnosis, as that will

be added to the narrative after the case has closed. This may be difficult, for example avoiding use of the term “blast cell” as this might be too specific, but describe the detail of the morphology of “possibly primitive cells”. The narrative and annotations are made available to a participant as soon as they have completed their submission; but the case may be open to others for at least 3 weeks. The scheme is not a proficiency test so BMS may, and indeed are encouraged to, share their experience and to discuss their findings with colleagues, but the ability to provide some immediate feedback is seen as essential by those taking part.

Annotating key morphological features on the image is time consuming for the case builder but must be done accurately and precisely. These annotations are the teaching element of the scheme and aimed primarily at those learning or trying to improve their morphological skills. The annotations must then be linked to the narrative so that as the description unfolds a click of a computer mouse can take the user to the exact cell being described and by hovering the mouse over the cell of interest an annotation displays extra information. In order to encourage experts to write narratives for cases the DM development team supported the creation and linking of the annotations, which then adds an extra step in the testing and checking procedures.

For the experienced morphologist the narrative supplies the information they need to see if it agrees with their own report but a trainee might then use all the annotation links to access the extra morphological information.

Once prepared both the narrative and annotations are checked by a second person familiar with building cases and agreed with the Consultant lead before being passed to members of the morphology SAG for testing and comment.

2.8.2 Professional validation mechanisms

As described in this thesis the operation of the DM scheme has a strict protocols for case preparation, validation prior to release, data handling and communication with participants, all of which is under the control of UK NEQAS(H) via the morphology SAG. This, in turn, reports to the UK NEQAS(H) steering committee. All processes comply with the UK NEQAS(H) policy for ethics concerning sample collection (Appendix A). Communication with participants is via UK NEQAS(H) or via the annual symposium for participants where there has been a feedback morphology session on the agenda every year since 2000. Complaints and suggestions from participants are reported back via regular meetings with the DM team and the annual morphology SAG.

2.9 Implementation of the UK NEQAS(H) digital morphology scheme for CPD

The launch of the world's first internet-based digital morphology scheme for CPD, aimed at individual professionals, was launched by UK NEQAS(H) in March 2008 with the first case, 0801DM, live in April 2008.

UK NEQAS for General Haematology

Digital Morphology CPD Scheme

Online CPD Participation

- Save the time and costs associated with travelling to and from an external CPD event
- Earn your IBMS approved CPD points online
- Access rare cases through high resolution digital slide images
- Option to use authenticated Wikipedia articles to assist you in your CPD answers
- User friendly online examination using Virtual Microscope Technology
- Submission of diagnosis and commentary online
- Secure online registration and CPD submission

Post CPD Submission

- Online CPD Scheme certification and portfolio printout
- Review case narratives
- Review annotated regions of diagnostic significance
- Visibility of group consensus

For further information please contact

UK NEQAS (H)
PO Box 14
Watford
WD18 0FJ
UK
Fax: + 44 (0)1923 217879
Email: haem@ukneqas.org.uk

Figure 2.8 UK NEQAS(H) leaflet announcing the launch of the DM scheme for CPD

Copies were distributed at the participants' symposia and sent to laboratory managers participating in the conventional glass slide scheme.

This unique scheme was designed to provide high quality images of blood cell morphology, set with an educational narrative, for laboratory-based professionals to

examine (Figure 2.9). Laboratories were encouraged to register individual participants rather than as a laboratory team. The scheme was accredited with the IBMS for CPD and participants gained a certificate for each case completed. It was anticipated that laboratories would be prepared to fund the cost of their own staff, as evidence that they were supporting CPD, costs for registration were to be kept at a minimum and UK NEQAS(H) offered laboratory managers a discount for buying registrations in blocks of 10 or more. As of 2015 managers became able to purchase DM scheme registrations as part of their routine annual renewal of EQA services. This streamlined the registration process for laboratories and made budgeting easier to manage. Importantly the administration software allows managers to see which of their staff are actively registered and who is completing cases, but they cannot access the registrants morphology reports, this meets the requirement of UK NEQAS(H) to retain the anonymity of participant reports. If the laboratory manager wants to see the printed reports from their staff, as part of an appraisal or evidence based practice, that remains an in house issue for the laboratory and is not influenced by UK NEQAS(H). Individuals may also register independently for the scheme via the UK NEQAS(H) website.

Participation rose from 219 individuals completing the first case (0801DM) to 603 registrants completing the second case and by the middle of the second year (2009) more than 1,000 professionals were regularly completing cases (Brereton *et al.*, 2010). All this occurred with very little active promotion. There was initial concern regarding the administration of the scheme and the workload for UK NEQAS(H) dealing directly with individual participant queries, rather than from just the laboratory manager, but the scheme needed to prove its longevity before staffing could be addressed.

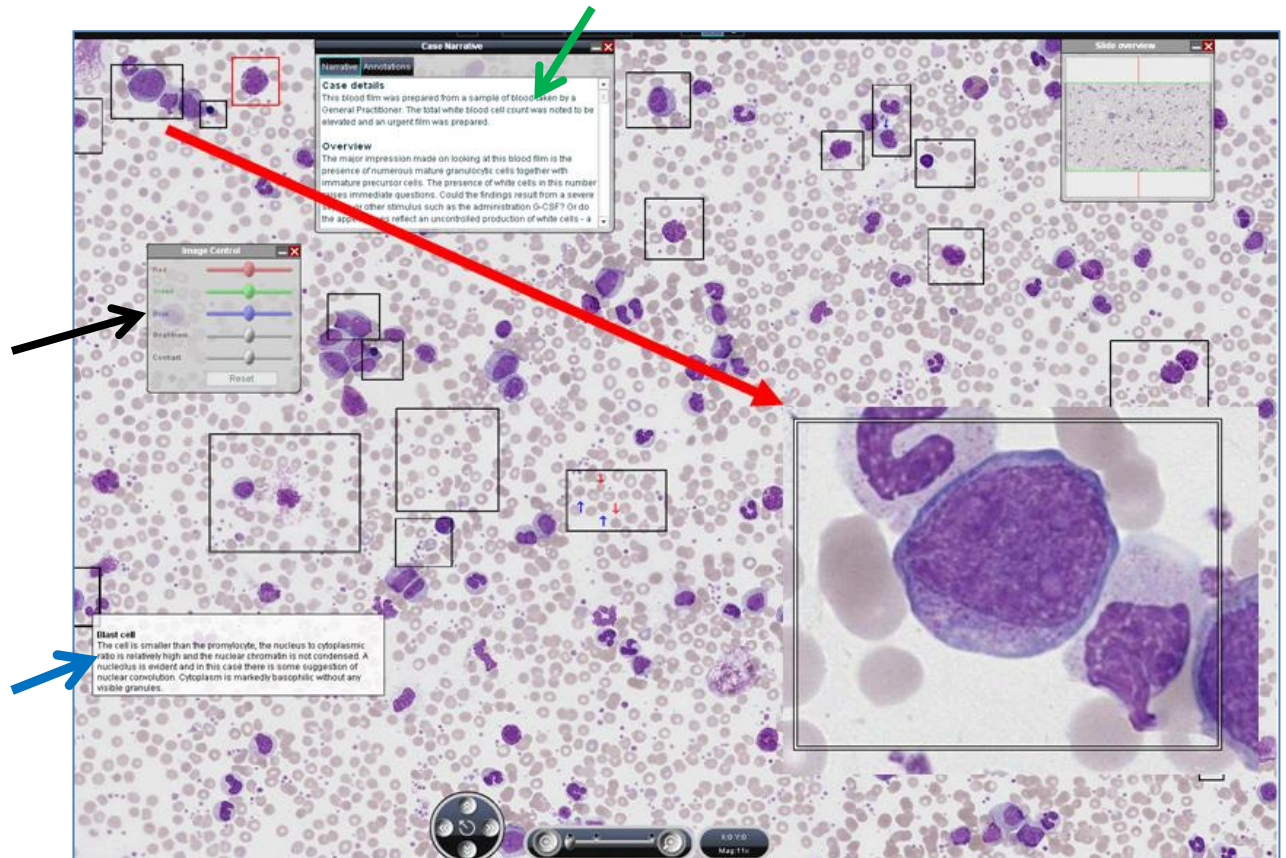
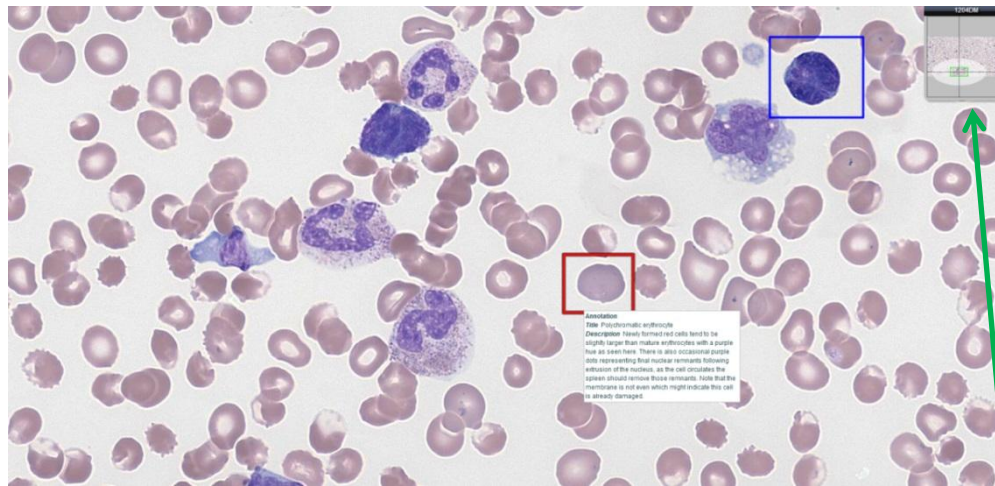
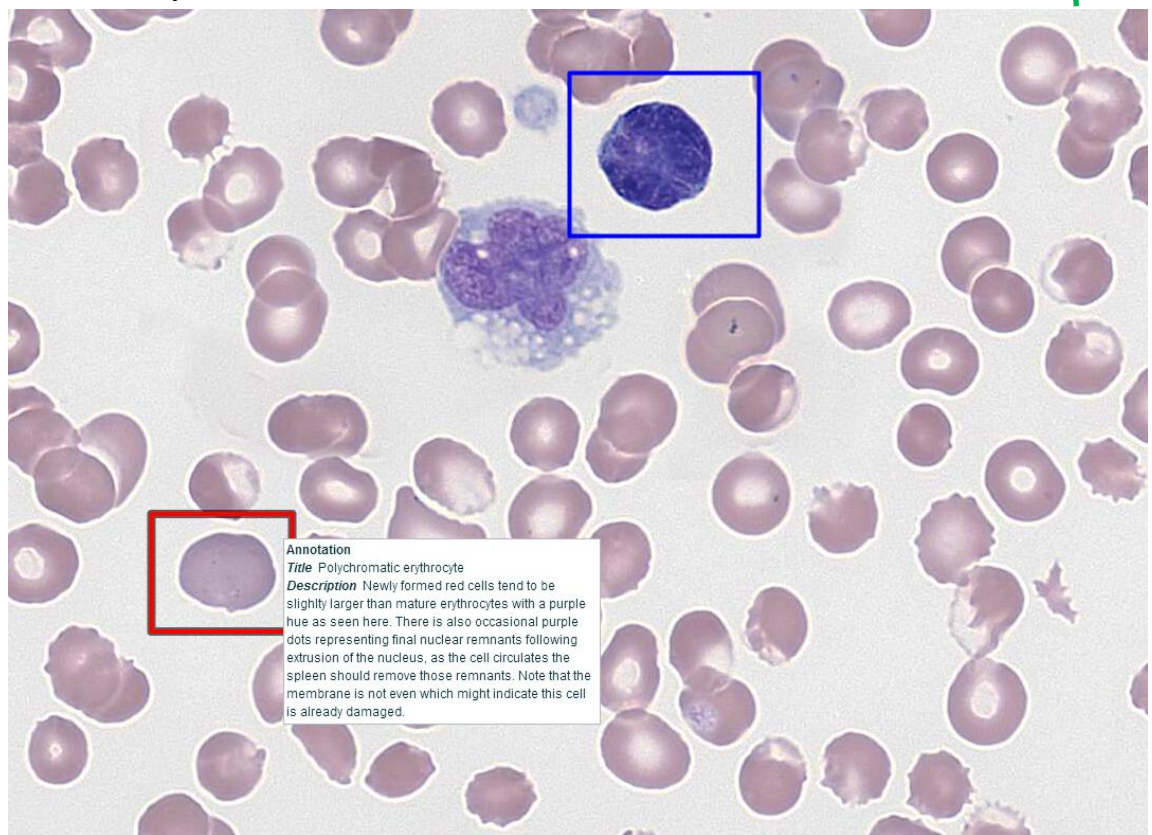


Figure 2.9 Composite snapshot of an actual case from the DM scheme. The red arrow indicates how the image looks at high power, the clarity of the nucleoli proving the quality of the original image. The black boxes show examples of morphology features that have been annotated by the expert which will show additional text when the user hovers the computer mouse as indicated by the blue arrow. The text box at the top (green arrow) shows the beginning of the narrative which has links to the annotations to aid user navigation. The grey box (black arrow) shows the tool available to participants to enable them to shift the colour balance to suit their own laboratories style of staining.

Further examples of image quality are shown in Figure 2.10 panels A and B, depicting one of the cases actually selected for examination in this thesis. It was important that the viewing software controlled the maximum zoom facility to avoid loss of image quality whilst still allowing the viewer to see nucleoli and cellular inclusions.



Panel A: snapshot from Case 5 in this thesis



Panel B: Snapshot at maximum “zoom”, Case 5 examined in this thesis.

Figure 2.10: Snapshots from Case 5 (UK NEQAS(H) DM 1204DM) Panel A: snapshot represents approximately one tenth of the image available for participants to view, the green box in the image map (top right corner indicated by green arrow) shows the location being viewed in relation to the total image. The red cell (box with red outline) shows attached annotation as would be seen after completion of a case as part of the educational aspect. Panel B: Maximum zoom allowed by the software to prevent pixilation of the image. Image taken with x63 oil immersion objective.

For all cases used in this thesis an example snapshot image and the original case description given to participants with the case at the time of participation are shown in Chapter 4.

2.10 Early evaluation of the UK NEQAS(H) Digital Morphology scheme for CPD

Recognising the level of commitment required by both Dr John Burthem and myself to the preparation of cases UK NEQAS(H) proposed to broaden the expertise of those writing educational narratives and, in so doing, increase the number of individuals able to build cases for the scheme. Members of the UK NEQAS(H) morphology SAG, who were already involved in testing cases before release to participants, were invited to undertake training for case preparation and in 2011 Dr Keith Paterson was the first SAG member outside the development team to provide narratives.

To strengthen the case selection process and to increase the opportunity to acquire suitable material DM scheme participants were asked to submit possible cases on a glass slide for consideration. As only one slide is needed the opportunity to use rare cases is increased although the first case to be selected by this method was an excellent example of a megaloblastic anaemia in 2011. As other morphology experts have volunteered to support the case preparation glass slide blood films are being reviewed by the team at the annual slide selection meeting held each year in January. A review of early cases showed that a good mixture of morphological disorders was being represented with both white cell and red cell disorders featured (Brereton *et al.*, 2010).

2.10.1 Participant feedback at the end of year one

The scheme ensured, by design, the anonymity of participants. Up to launch the team had used the annual exercises to develop the system but once the scheme went live the early feedback from participants was via individual queries to UK NEQAS(H) and was mainly anecdotal. After one year UK NEQAS(H) circulated a questionnaire to all participants (n = 649), 411 completed returns were received, of which 49% stated they

were BMS, 36% were senior or chief grade BMS, 11% said they were in a primarily managerial role and the remainder (4%) were either trainee BMS, clinical scientists or medical staff (Eke *et al.*, 2010).

Whilst responses about the actual cases were positive with more than 95% appreciating the quality of the images and the range of morphology, the team were interested in problems around the access and completion of cases and the needs of participants, 27% said they had contacted UK NEQAS(H) with queries. Of those regularly accessing cases around 5% did not actually submit before the deadline and nearly 40% had failed to complete all the cases they had access to.

Reasons given by participants for not completing cases (Eke *et al.*, 2010):

- Time constraints
 - annual / sick leave
 - unaware / forgot deadline
 - poor staffing at work / no time allowed
- Computer and IT issues
 - Trust network problems
 - Struggle with Slidepath™ software for data entry
- Lack of confidence with the morphology
- Forgetting, lack of reminder
- Late registration

Following the feedback the team introduced several developments including the emailing of all participants with information on case closure dates and reminder emails one week prior to closing. The team also concentrated with Slidepath™ on the data entry and handling experience and key performance indicator targets were introduced to be monitored at the regular telephone meetings. To help with registration problems for new users an on-line video was added with visual demonstration of how to complete a case.

Of note was the response to the question on the level of reporting skill the participants perceived themselves to be, with 13.8% reporting films outside of core hours where advice or support from colleagues would be low. These were exactly the people the scheme was aiming to support.

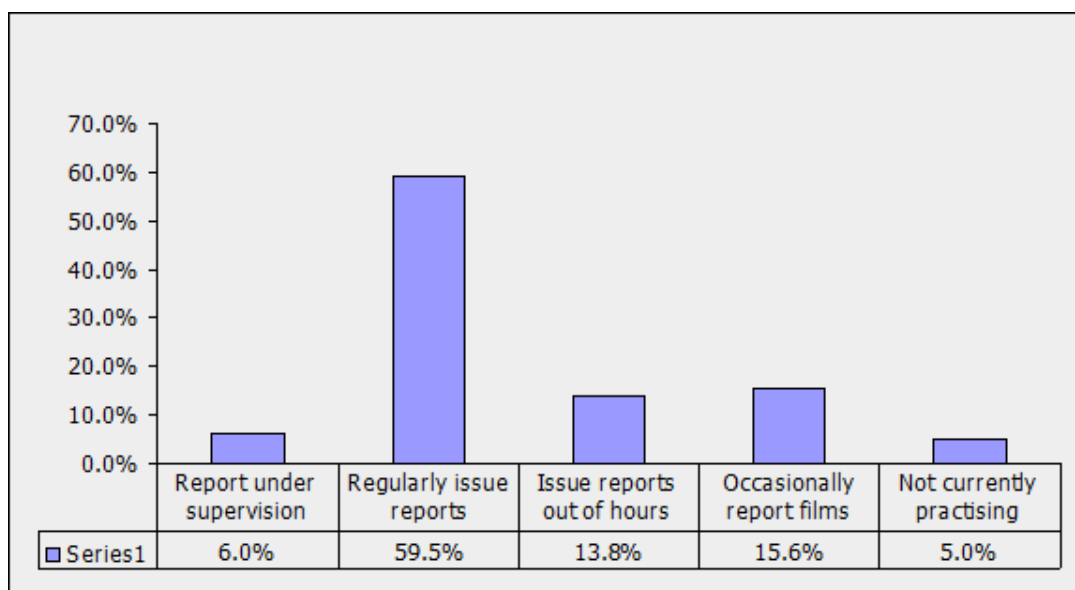


Figure 2.11 Responders statement on the frequency of their film reporting. Bars show the self-assessed morphology reporting level of responders (total = 411) who regularly complete cases on the morphology CPD scheme. (Eke *et al.*, 2010).

2.10.2 Workshop at the end of year two

At two years' experience, with a dozen cases released, around 2,000 individuals registered and over 1,200 regularly completing cases the team wanted to know how successful the current cases had been in meeting the needs of participants and what should the priorities be going forward so that the scheme continued to thrive.

At the annual UK NEQAS(H) symposium October 2010 in Birmingham a workshop was held (led by Dr Burthem and myself) to review the standing of the DM scheme and to gain more information from participants and laboratory managers on their concerns and expectations. As scheme participation is anonymous we had no knowledge of the skill level or experience of participants (junior, non-practising or expert), so the workshop aimed to gain feedback from users and also from those who had chosen not to register. UK NEQAS(H) issued additional specific invitations to participants from whom they had received particularly strident comment or negative criticism as the DM team were particularly interested to address any issues that were detracting from the schemes appeal. Prior to the workshop a questionnaire was prepared and issued to 99 registrants, the 75 completed returns used as the basis to drive discussion. The entire process for accessing and answering cases by participants was walked through at the

workshop in an interactive session to allow the ninety-nine attendees to input and critique the process independent of whether they, or their staff, were participants (Brereton *et al.*, 2011).

Regarding complexity of the cases 70% of responders believed the DM scheme was meeting their expectations, during discussion some felt that simple cases such as iron deficiency should be presented more frequently, whilst accepting that the scheme needed to appeal to both trainee and experienced morphologists. This feedback strengthened criteria for case selection; cases needed to contain morphology that will not put off the novice but will also appeal to the more advanced practitioner. To achieve this a mixture of cases is needed of which some need to contain something beyond that which may be routinely found.

The educational narrative attached to cases scored very highly with both participants and managers, and the professional validation of cases prior to their release was seen as a value added component with 95% of responders scoring this as useful (Figure 2.12).

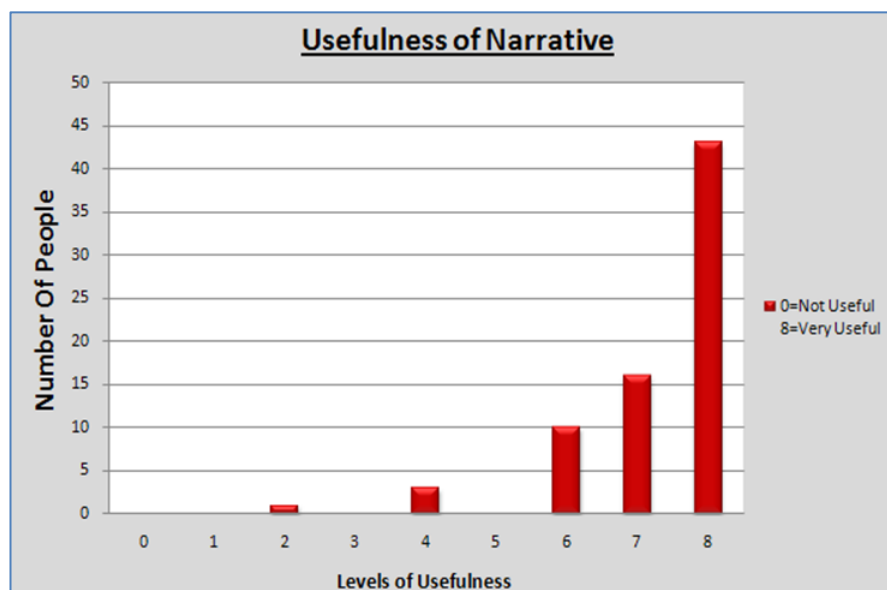


Figure 2.12 Usefulness of narrative as scored by workshop responders. The majority of responders (n = 75) considered the educational narrative assigned to a morphological case as useful. Valued of 0 to 8 where 0 = not at all useful, 4 = useful, 8 = very useful. (Brereton *et al.*, 2011).

Three responders (2.3%) had a negative opinion of the schemes impact on training for morphology skills (graph not shown) but did not elaborate. Encouragingly 93% stated that it had increased their own interest in morphology (Figure 2.13). That the scheme had raised the profile of morphology within the laboratory amongst staff might not be measurable but it was seen as a positive outcome by the majority.

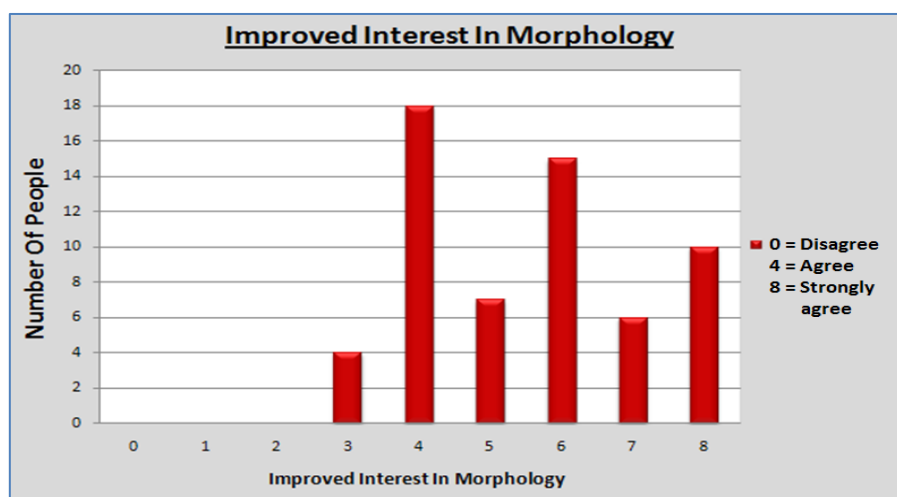


Figure 2.13 Improved interest in morphology as scored by workshop responders. Responders (n = 75) gave an agreement score (0 to 8) on the statement that completing cases of the DM scheme had improved their interest in the subject of morphology; where 0 = strongly disagree, 4 = agree, 8 = strongly agree. (Brereton *et al.*, 2011).

When asked about training 71% believed that the scheme produced a novel morphology resource for individuals and discussion from training officers within the workshop suggested that as the case list grew the back catalogue of annotated cases would provide an important library for morphological education.

It was noted that only 8% felt strongly that the awarding of CPD points was the main value of the scheme and importantly the vast majority saw the main benefit being the maintenance of their morphological skills (Figure 2.14). The DM project team had seen the accreditation of the scheme with the IBMS as a key attraction for potential participants so it was encouraging to realise that those participating acknowledged an actual professional benefit beyond the collection of CPD points. Since 2010, the demands of inspection bodies that individuals show evidence of CPD has substantially increased and the DM scheme continues to acknowledge the importance of the educational aspect in case preparation.

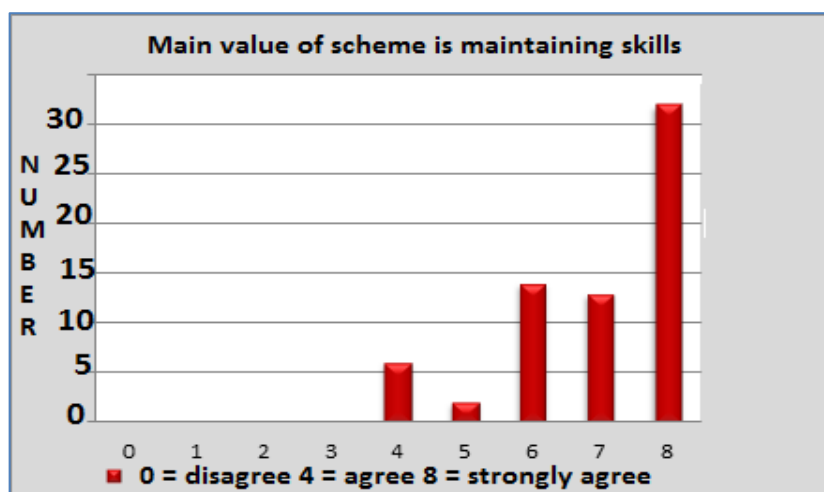


Figure 2.14 “Do you see the main value of the DM scheme to be maintaining morphology skills?” Responders (n = 75) gave an agreement score (0 to 8) on the statement that the main value they received from participating in the DM scheme was that their morphology reporting skills were maintained; where 0 = strongly disagree, 4 = agree, 8 = strongly agree. (Brereton *et al.*, 2011).

Discussion about the style of MCQ was positive, there was considerable debate over the style of question, feedback suggested participants liked the questions that made them consider their morphological findings and offered an element of interpretation. (Figure 2.15). There were no suggestions on how that might be improved.

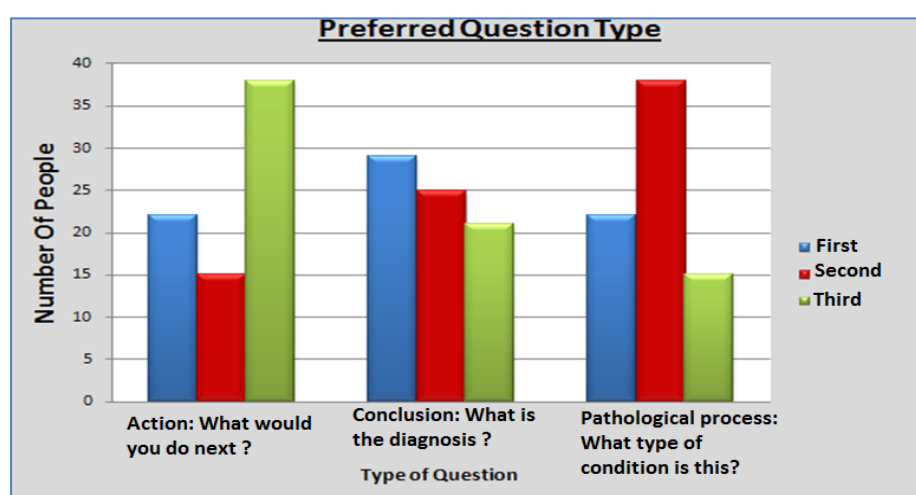


Figure 2.15 “What is your preferred type of MCQ from the 3 most used at this point?”. Participants were asked to give their preferred style of MCQ and to give their second and third (least favourite) option. Choices of MCQ style were based on Action, Diagnostic summary (conclusion) or Pathological process. (Brereton *et al.*, 2011).

It was, however, accepted that the question would be set by the DM team dependant on the case. Knowing whether participants considered certain findings warranted

urgent or routine referral was something the DM team felt might be important to know. It was encouraging to have participants engaging with the process so positively about the pathological and clinical aspects of their DM reports.

2.11 Examining a stitched image: the viewing experience

Alongside the feedback from the workshop and from the participants who contacted UK NEQAS(H) directly, the team also had the information in the reports completed for each DM case. If participants did not report key features or did not identify the pathological condition correctly there were many possibilities; skill levels were not known, equally the team did not know how participants approached examination of morphology on a PC screen. Indeed the question of how users approach and examine stitched morphology images on a computer remains largely unstudied as there were no equivalent schemes in operation.

Biomedical scientists are taught to examine blood films on glass slides using a standard protocol such as the “battlement” approach, generally accepted as the logical way to approach viewing films via a microscope (Bain, 2005). Even so, they may choose to ignore or avoid cells they are unsure about (Houwen, 2001), which is more difficult to do when viewing a preselected stitched image. A microscope provides both physical and mental constraint in that the field of view is finite and controlled.

- A blood film covers most of the glass slide, a defined field of view can be seen at any one time, so if the microscopist has to seek to find certain features they do not feel pressured. For DM, however, if a large image is presented the viewer may feel disheartened by the sheer size of the area they are presented with or simply unsure where to start.
- It is unnecessary to view an entire film every time by microscopy, so the fastest method of seeking the correct view is needed. The microscopist seeks an area where the red cell are just touching but not completely overlying each other. This is not the case for a digital image where the area of cells to view has already been selected and presented.
- As cells are distributed unevenly in a blood film, with larger white cells possible dragged to the edges of the film the viewer starts close to one edge of the film

and moves across, so as to get the correct representation of smaller and larger white cells. Again this is probably not required in an image of limited size where the user can quickly navigate and will know exactly where they are. It is essential; therefore that the person creating the image has considered what cells must be present within it.

- Looking down a microscope the magnification of view is tightly controlled, it can be increased or decreased to set levels by changing the objective but the microscopist usually will remain at one level of magnification for most of the examination. This does not apply to viewing an image where the use of a “zoom” facility enables the viewer to change the magnification smoothly and with ease at any point.

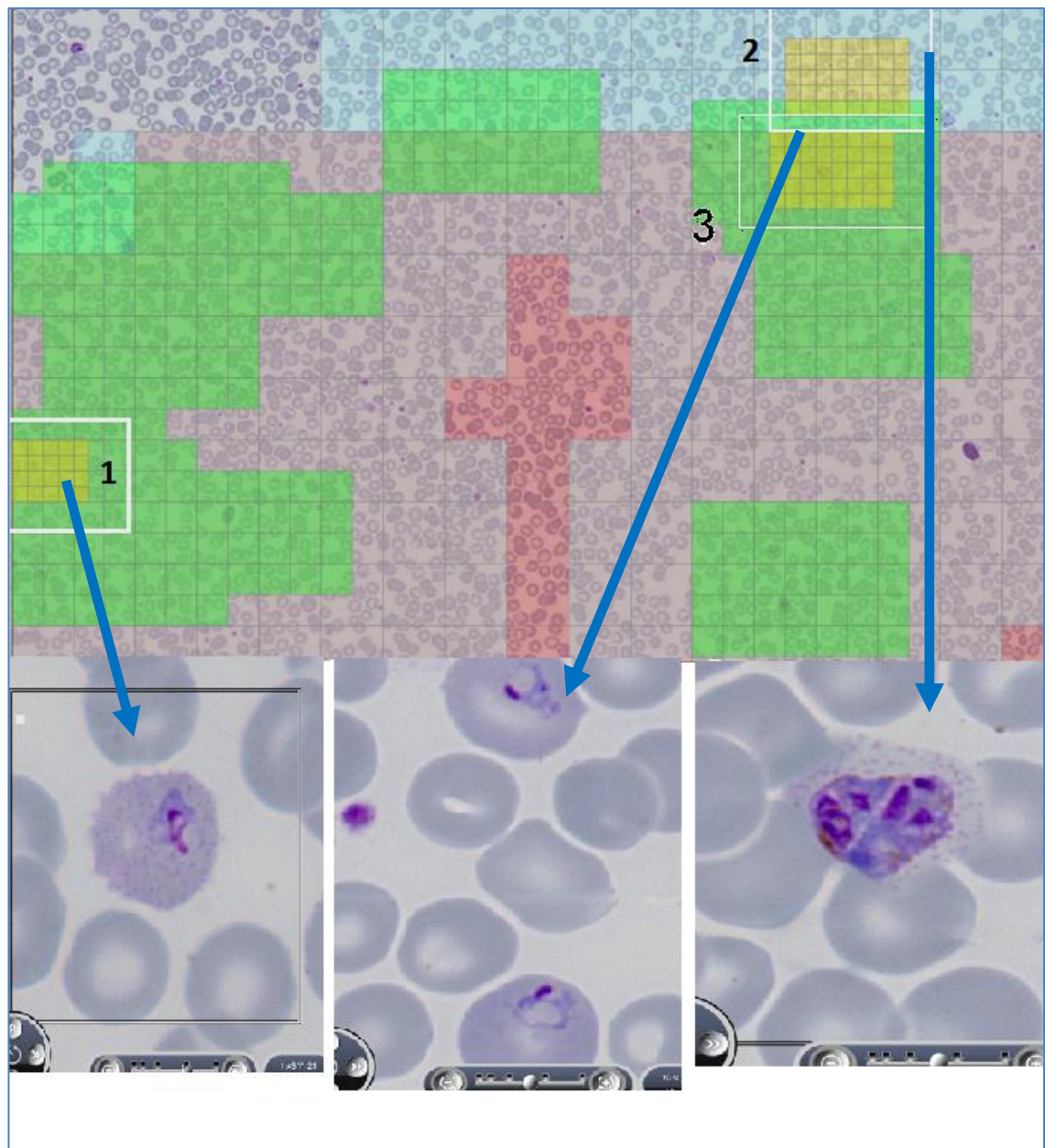
All these aspects remain fairly untested and must be considered by those presenting morphology images to users.

2.11.1 Examining a stitched image: actual practice

The way people view digital images has been studied showing a different approach to that taken using microscopy (Raghunath, Braxton & Gangnon, 2012). User-tracking software was not part of the DM scheme so testing the approach taken by participants to viewing images was not possible. With the support of Slidepath™ a version of the same software but with user-tracking facility was made available for an exercise designed specifically to consider how users view DM on a PC screen. The software would trace the movement of the image in view, record the zoom views and record the time spent on each view and the magnification used. This information would then be stored as ‘heat’ maps which could be laid over the image retrospectively to show where the users had been viewing and how long they had spent on an area. Two cases were prepared with large scale images exactly as prepared for the DM scheme; one a malaria species *P. ovale* and the second a case of Burkitt’s lymphoma. Both had already been circulated by the conventional UK NEQAS(H) glass slide scheme and had also been used in small scale digital format (40 field images) in exercises for UK NEQAS(H) participants (the *P. ovale* case formed part of the 2004 exercise detailed in section 1.8.2). The new exercise was trialled at the International Society of Laboratory Haematology (ISLH) congress in Sydney, Australia in

2008 and was completed by 134 individuals over three days. The results were compiled for presenting back at the meeting as a lecture on the final afternoon. The audience was different to that regularly participating in the UK DM scheme with the majority being clinical (medical doctors) rather than technical scientists or BMS, additionally the majority were non-UK based so unfamiliar with the DM scheme.

This was an informal, interactive event for congress attendees from various countries and clearly not a controlled scientific trial, the viewing software mapped the experience of the user, where they looked and how long they spent on each area. Despite the informality of the exercise, the substantial number of enthusiastic attendees attempting the exercise made it interesting to see how different the viewing patterns were compared to the standard expected approach to microscopy. Of those who took part in the exercise no-one used the standard “battlement” approach to viewing the images, instead users “zoomed” to high magnification directly on features they thought might be interesting from the low power view. They zoomed to high and low resolution to move across to find features of interest but rarely viewed the entire image. The heat maps showed where on the image users concentrated their attention, in most cases this was on just a couple of key features before reaching their conclusions; possibly because they were experienced or felt they had sufficient information to make a quick decision or possibly because this was a “fun” exercise and lunch was calling. The heat maps below are from three individuals who completed the *P. ovale* case and are typical of the user viewing experience; these demonstrate the realities and pitfalls of viewing morphological images via a computer.



Area 1: fimbriated red cell with trophozoite. Area 2: shizont infected red cell. Area 3: two trophozoite infected enlarged red cells.

Figure 2.16 User A: Heat map and the parasites most viewed. Outcome correct identification of *P. ovale*

The heat map overlies the entire stitched image presented for the *P. ovale* case.

Heat map area colour codes: **Cerise** = Not viewed at high power. **Lilac** = Scrolled across area but did not spend time stopped. **Green** = spent more time on area or viewed area more than once. **Yellow** = spent more time on this area or repeated viewing of same area.

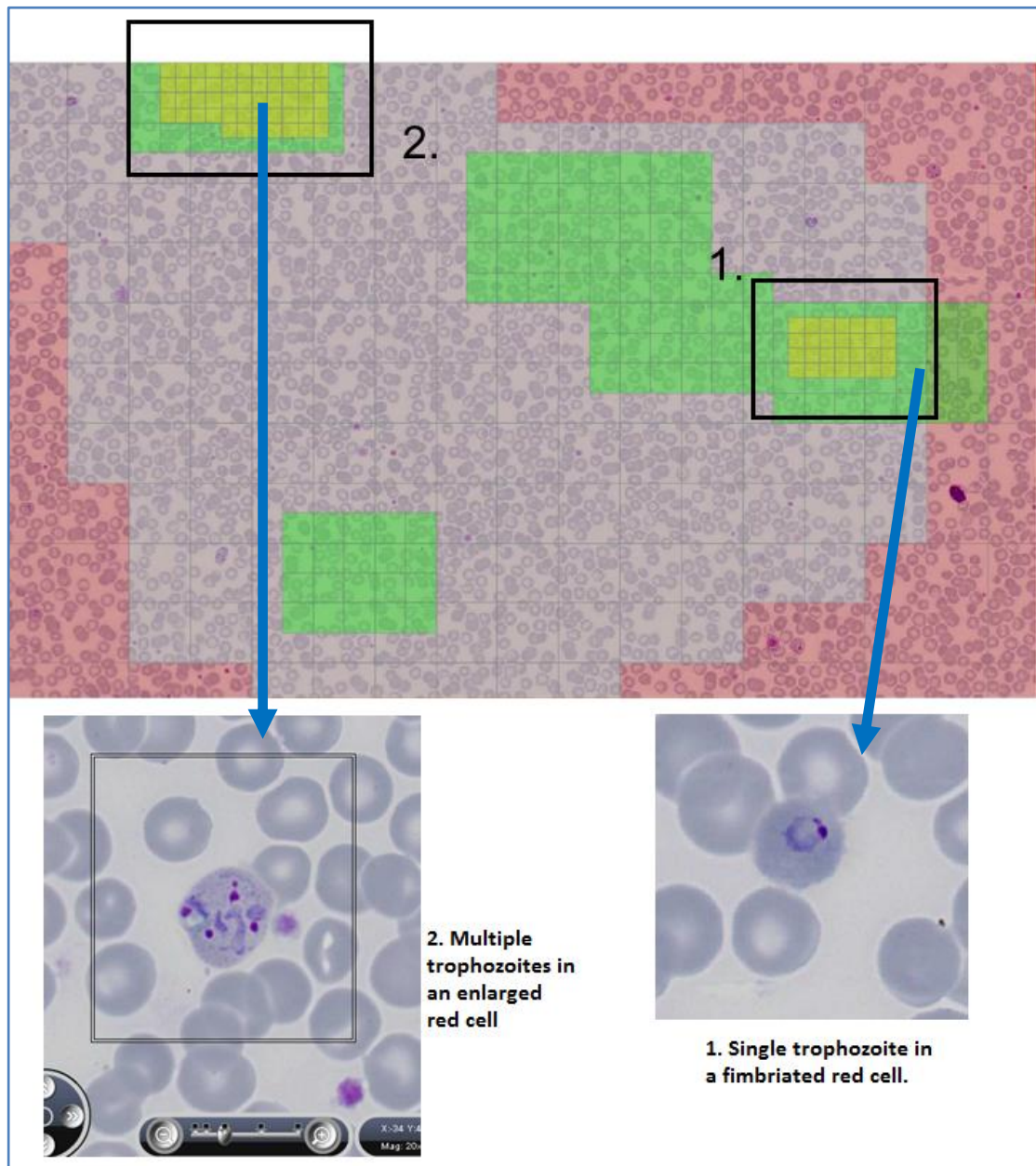


Figure 2.17 User B: Heat map and the parasites most viewed. Outcome incorrect identification of *P. vivax*

The heat map overlies the entire stitched image presented for the *P. ovale* case.

Heat map area colour codes: **Cerise** = Not viewed at high power. **Lilac** = Scrolled across area but did not spend time stopped. **Green** = spent more time on area or viewed area more than once. **Yellow** = spent more time on this area or repeated viewing of same area.



Figure 2.18 User C: Heat map showing areas viewed with at least 8 key features examined (areas in yellow). Outcome incorrect identification of *P. falciparum*

The heat map overlies the entire stitched image presented for the *P. ovale* case.

Heat map area colour codes: **Cerise** = Not viewed at high power. **Lilac** = Scrolled across area but did not spend time stopped. **Green** = spent more time on area or viewed area more than once. **Yellow** = spent more time on this area or repeated viewing of same area.

Despite not viewing all of the image available, and only examining 3 infected red cells in depth (yellow blocks Figure 2.16), user A came to the correct morphological diagnosis. User B examined less of the image coming to an incorrect conclusion of *P. vivax* (Figure 2.17). The species of *P. vivax* and *P. ovale*, however, are similar in morphological appearance, there was no additional clinical information to aid diagnosis and only viewing two parasite infected red cells may have proved insufficient to reach the correct outcome. User B did not examine any comet shaped infected red cells, which were present and might have positively influenced the outcome. User C spent a lot more time on the image and examined nearly every parasite infected red cell present (yellow blocks Figure 2.18), despite this user C then reported an incorrect

outcome of *P. falciparum*. Having viewed sufficient key features to negate this outcome it might be that user C had limited experience of malaria morphology. So the problem remained that without knowledge of the skill level of the user it is difficult to measure the success of process, but the exercise supports the concept that user approach to digital morphology is different to the user approach to microscopy just as the opportunities, constraints and limitations are different. If the approach is so different there is concern that information gleaned from studying responses to DM might not be transferable to morphology education using glass slides. Having used the malaria case both in the UK NEQAS(H) glass slide scheme and in a DM exercise (Chapter 1.8.2 Figure 1.8.) where the outcomes appeared similar, it was interesting to add the outcomes from this larger image, viewed by professionals in an international setting. Figure 2.19 shows this data and suggests that the outcome is comparable from DM and microscopy, as the same proportion of users (< 40%) attained the correct diagnosis.

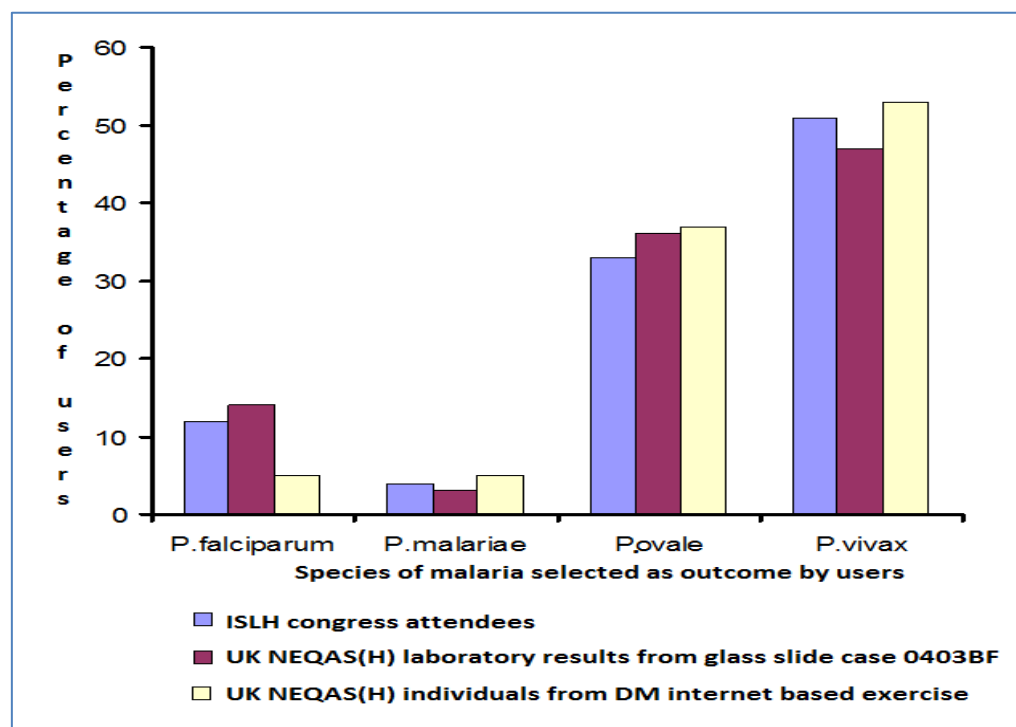


Figure 2.19 Comparison outcomes of species of malaria selected by different professionals viewing a case of *P. ovale* in different formats. ISLH attendees (n = 134) provided large stitched image, UK NEQAS(H) participants (n = 256) provided glass slide and UK NEQAS(H) DM exercise participants (n = 162) provided small scale stitched image. Individuals selected the species of malaria they identified as present. Correct outcome was *P. ovale*. (Presented as Conference oral presentation at the ISLH, Sydney, Australia, 2008; Brereton and Burthem).

Although fewer users (71 of 134) completed the second case the responses to the image of Burkitt's lymphoma also mirrored outcomes from previous UK survey data. It is not known whether users found this case more challenging or less interesting than the malaria case. With the addition of an MCQ asking what immediate actions the user would take (similar format to the UK NEQAS(H) DM scheme) that had not been part of previous exercises for this case, it was noted that those who had diagnosed the case as viral infection (30%), rather than lymphoma, were less likely to have selected that the case be sent for urgent referral.

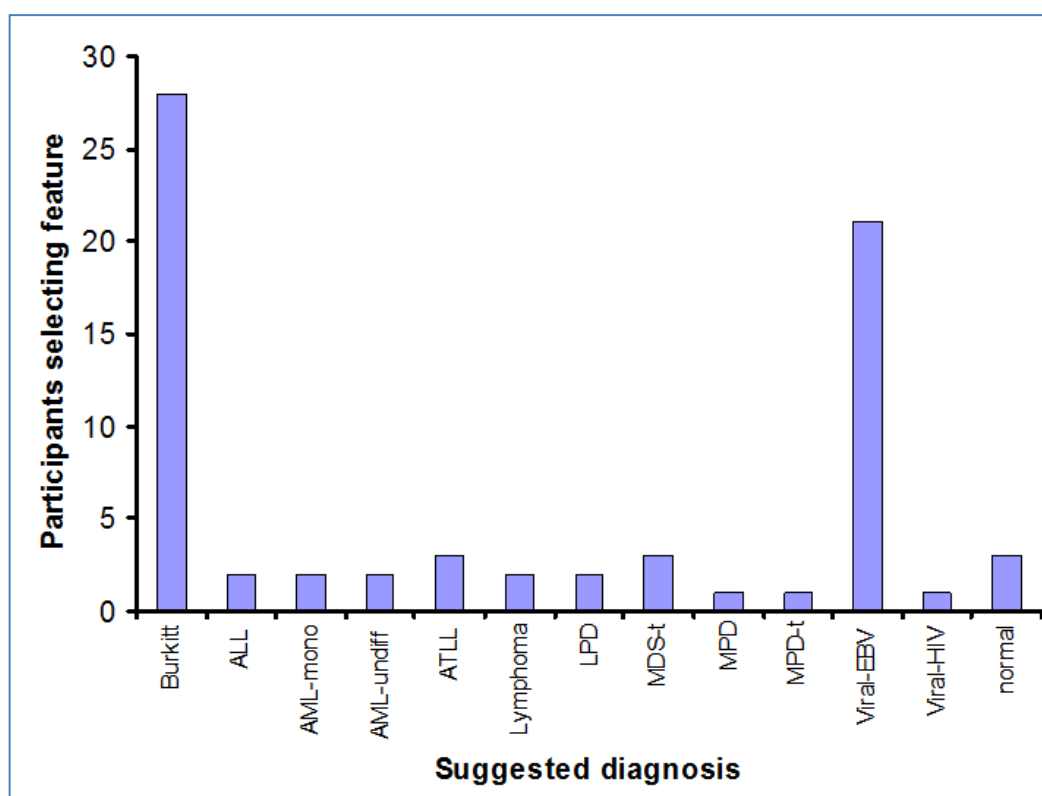


Figure 2.20 User outcomes for the Burkitt's Lymphoma image at the ISLH congress Australia 2008. ISLH participants (n = 71) outcome diagnosis selected by individuals. (Presented as Conference oral presentation at the ISLH, Sydney, Australia, 2008; Brereton and Burthem).

This was the first time that an exercise linking the morphology report to an outcome action had been considered by professionals at an international meeting. It is a real concern that clinically significant findings might be missed if a wrong conclusion is drawn.

2.12 The UK NEQAS(H) DM scheme for CPD at 4 years: proposal to examine participant responses

This chapter has described the successful introduction and early analysis of the UK NEQAS(H) DM scheme for CPD. By March 2014 a total of 34 cases, approved by the UK NEQAS(H) Morphology SAG had been issued to, and examined by participants. Completion of cases ranged from 219 to 1318 individuals, median 995 (details of surveys issued with diagnosis and completion rate see Appendix C).

2.12.1 Current status and future requirements

The criteria put in place for case preparation continued to be employed as originally agreed (section 2.5) and generally by the same small team. Although feedback mechanisms remained in place and Dr Burthem and myself presented at every annual UK NEQAS(H) participants symposium, there was a wealth of data that had not been considered. Case returns were examined in order to prepare the consensus reports for participants, as part of the schemes function (Brereton *et al.*, 2012), but an in depth examination of the data being generated by participants had not been undertaken.

2.12.2 Evaluating the reporting process to inform future direction

The blood film report is still the final arbiter when abnormal results are found in a FBC. Digital morphology has opened a world of tools available for education and support, but the interpretation of the morphological features still requires experience and skill. The interpretive element of the examination and the resultant actions can have significant clinical impact but these have not been widely studied and are not understood. In a time pressured laboratory the need to prove competency is ever present, so it is important that the different skills sets required to produce a useful morphology report are investigated. Professionals need to understand what errors occur in the decision making process and whether this could be improved using the DM scheme.

It might be assumed that once trained to take a standardised approach to reporting a blood film via microscopy that this technique would always be applied. There is no published evidence to support this (Sinclair 2005) and no major studies of how blood film reporting is completed. We have shown that the approach adopted for examining

digital images does not reflect those used via microscopy, however, the ability to recognise morphological features and the skill of distilling those into a final report are essential, whether that be by microscopy or by examination of a digital image.

It might also be assumed that the quality of reporting morphology improves with experience and that the interpretive skills of individuals likewise improve. Equally, that errors in interpretation and reporting reflect lower skill levels and that this may reflect inexperience and a lower level of knowledge. However, as morphology reporting has not been examined on a large scale the types of error that occur are unclear.

The DM scheme had not been designed to evaluate the decision making process required to produce a report. The structured format of the cases, however, meant that they were examined in a standard process and as large numbers of professionals participated suggested that data generated from their reports might have value.

Structured format of the participant experience:

1. identify and select important morphology features
2. rank the features in priority order
3. interpret features to answer the MCQ on action to take
4. free format question to interpret the features in to a pathological process or morphological diagnosis

2.13 Proposal to evaluate participant data to test the association between feature selection and report outcome

Aim of the evaluation as proposed to the Morphology SAG:

Evaluation of the case data to inform the priorities for development of the DM scheme required to take it forward over the next 10 years and to determine if the educational narrative could better support skills for reporting morphology. Evaluate whether participants produce informative reports from the images they were presented with.

The proposal to evaluate participant reports was an attempt to answer these questions with a view to setting future objective for how the scheme might progress and reach new users. As a minimum outcome this was an opportunity to consider any changes needed to keep the DM scheme relevant to current participants.

2.13.1 The questions for my research are:

- To examine how participants reach their interpretive conclusions from the observations they record.
- To examine whether the conclusion participants reach influences their subsequent actions.
- To identify successful strategies used to produce a correct and informative report.
- To identify sources of errors made by participants when producing their reports.

2.13.2 Ethical considerations and governance of research

No patients were to be bled for this work, all case material for the DM scheme was already controlled by UK NEQAS(H) procedures. The very nature of the schemes data collection system ensured no participant data could be linked back to an individual and data extraction would follow the same governance system as data supplied by Slidepath™ required to complete the case reports for the DM scheme. Governance of the research came under the scope of the DM project started in 2000 and would be controlled by the Schemes Director and Manager, via the Morphology SAG with the added provision that should there be any evidence that the research would put either the anonymity of participants or the reputation of UK NEQAS(H) at risk the project would be stopped (Figure 2.21).

Ethical issues considered by both the Morphology SAG and by UK NEQAS (H) management and was approved by them as not requiring further application 18th June 2011 under two main categories:

a/ The preparation of morphology cases as covered by the policies applied to the collection of all material for EQA purposes in that samples may only be used if they are discard material from routine testing which has been fully anonymised and is not traceable back to a patient. For the use of images in the digital morphology scheme clinical details and patient demographic data are also altered from the original, *i.e.* patient ages and test results are altered from the original source data.

b/ The extraction of participant input from the CPD scheme is totally anonymised and not traceable. It is not possible to associate extracted data to any individual. No participant demographic data will be extracted nor made available. The participants are not being tested, this data extraction is to interrogate the scheme design and ensure it is meeting objectives.

A clear governance pathway was proposed to ensure the research remained within approved remit. My commitment is shown in brown and that of my internal supervisor in blue below:

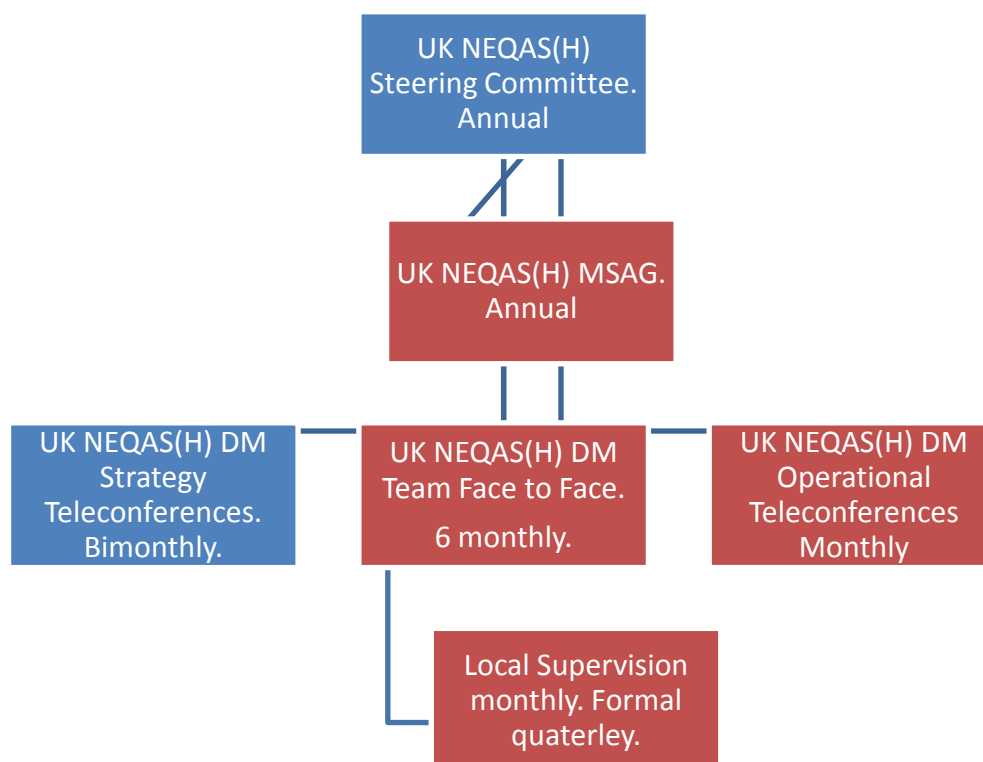


Figure 2.21 Governance of research.

Chapter Three: Methods used to evaluate participant responses

3.1 Selection of DM cases for data examination

The morphology depicted in the cases presented for the UK NEQAS(H) DM scheme varied in complexity and was dependent on the clinical conditions represented. This variety was deliberate as the scheme aimed to appeal to participants of all skill levels from those in training to those with many years of experience reporting morphology. It was important to select cases for examination that represented the morphological diversity occurring in laboratory practice and that also challenged the skill sets across all levels of experience. The cases selected tested the widest possible range of morphological reporting skills representative of:

- A. A single but distinctive morphological feature (related to white cell lineages) that indicated a specific diagnosis of clinical importance for patient treatment.
- B. A combination of morphological features involving different cell lineages which demanded more complex interpretation but which depict combined clinical conditions that are directly related and might be expected to be found together.
- C. Complex case involving abnormalities in different cell lineages and which represented more than one significant pathological process occurring independently. These were separate and unrelated clinical conditions occurring simultaneously that would not be expected to be found together.

The process for this examination set out below was agreed with the morphology SAG and the UK NEQAS(H) Scheme Manager and Director. The selection of the specific cases was agreed with the consultant lead.

At the time of case selection the only cases analysis data already published to participants was that which formed part of the consensus DM scheme report.

Table 3.1: Basic details of cases selected for examination.

Complexity of morphology	Case number of study	DM scheme survey number	Morphological diagnosis	Number of participants
Single distinctive feature	1	0902DM	Viral infection (glandular fever)	732
Single distinctive feature	2	1206DM	Pelger-Huët anomaly	1,108
Complex: Related combination	3	0903DM	Microangiopathic haemolysis plus viral infection (HIV)	752
Complex: Unrelated combination	4	1201DM	Haemoglobin SC with acute myeloid leukaemia	1,011
Complex: Conditions related by an external contributing factor (therapy). Skill level known	5	1204DM	Oxidative haemolysis (G6PD deficiency) with adult T-cell leukaemia/lymphoma	789

3.2 Data extraction, coding and methods of analysis

The case data requested from Slidepath™ were extracted from the secure database and arrived as XML files without participant identification information. A separate excel spread sheet was then assembled for each case and the data imported, there was no link of participant identification between cases.

For each case every participant data set included:

- A time stamp number to link each participant's responses across the various elements within that one case. (No link to an individual's responses between cases).
- The morphological features that the participant had selected, up to a maximum of five, listed in order of the priority they had chosen *i.e.* 1 = most important, 5 = least important.

- The MCQ answer selected by that participant for the associated question (generally the outcome action they would take).
- The free text (characters unlimited) interpretative element at the end of the participants report for morphology diagnosis.

Each participant's data set had to be manipulated to align the information on the spread sheet prior to coding for analysis. Submissions that had corrupt, ruined or incomplete data sets were removed prior to analysis *i.e.* partial submissions where individuals had not fully completed their report by the closing date of the case (for example did not complete the MCQ). Numbers are given in the results section for each case.

Coding strategies are detailed below and were developed for all points in agreement with the Lead Morphologist (Dr John Burthem) and verified by the UK NEQAS(H) Director (Professor Keith Hyde).

3.2.1 Coding morphological features

The morphological features selected by participants had been chosen from a set of options which were standardised across all cases. Participants were permitted to select any combination of red cell, white cell and platelet features or from just one lineage.

The morphological features they selected were then coded to allow statistical analysis and comparisons of red cell, white cell and/or platelet choices. White cell codes were assigned the prefix 1XX; platelet codes 2XX and red cell codes 3XX so that cell types could be compared. Participants were asked to prioritise up to a maximum of five features from the 74 options available to them, these are shown in Appendix D.

Once coded the morphological features selected by each participant were kept in separate columns on the spread sheet to maintain the priority rank assigned to them, so that the importance of features was not lost during manipulation and could be used in the analyses. Where participants had selected fewer than five features their selections were ranked from one downwards with remaining fields being coded as zero entry. During analysis the assigned priority enabled those features given high priority (one and/or two) to be separated from those given less important weighting. It was

anticipated that for simple cases some participants would not need to select five features to complete their report.

Where a single feature was considered of prime diagnostic significance *e.g.* Pelger neutrophils, the feature was coded as a single data element. For the complex cases, with multiple individual morphological features across all cell lines, there were several feature options that would be considered analogous or represent a similar outcome. Where a combination of features indicated one pathological process (*e.g.* microcytosis, hypochromia and pencil cells may all be present in a case of iron deficiency) following initial coding, the elements were placed in to feature-groups to represent that associated process. This condensed the large number of related features selection for analysis and was expressed as the mean number of features selected (from a maximum of five) with standard error of the mean (SEM).

A “priority score” was then generated from the rank the participant had assigned each feature: for single elements *e.g.* Pelger neutrophils this was the priority rank assigned by that participant, for feature-groups this was the highest rank given by that participant for any feature of that group.

Statistical evaluation was directed by Dr Burthem and employed GraphPad Prism software (v6.04) using contingency table analysis to perform a comparison of morphological feature selection or diagnosis employed (Chi-square test: Fisher’s exact test, two-tailed T test for means assuming unequal variance). Priority scores for frequency of choice were compared between multiple groups using a non-parametric ANOVA test (Kruskal-Wallis test with multiple comparisons of means). For two sets of observations a two tailed Mann-Whitney test was employed. Significance is indicated on bar chart figures as follows: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.

3.2.2 Coding the multiple choice question

The answer selected by participants from the MCQ had been from pre-set options specific to each case. Although the phrasing of the MCQ questions varied for each case they generally asked the participant to select a course of subsequent action based

on their findings and the interpretation of the conclusion they had reached. The answers were then coded A to E for analysis with A being the ideal or expected action and E being the most inappropriate action or detrimental in terms of patient care. The MCQ options for each case are shown in Chapter 4.

For each case the MCQ answers were assessed against the morphological features selected by the participant, the priority assigned to those features and their free text responses attributable to morphological diagnosis or outcome. The action taken by the participant was a key element indicating the level of urgency or clinical significance they associated to their findings.

3.2.3 Coding the free text interpretative comments: morphological diagnosis

Coding of free text responses presented the greatest challenge to the analysis of data. The morphological diagnosis submitted by participants was in unlimited free text, which produced a vast range of possibilities with participants using combinations of varied terminology and acronyms along with reflective comments on their reaction to the case or evidence of their own further reading on the subject. Participants would also discuss features they did not find to show they had considered or excluded certain diagnoses so word recognition software could not reliably be used.

In total over 4,300 free text comments were examined, distilled and a coded option applied, this was a labour intensive exercise as all comments were checked more than once. The free text question had been different for every case so the treatment of the answers for each had to be considered independently. In order to achieve this and to reduce bias, the first 100 answers of each case were examined and the common outcomes considered for coding. The initial coding was undertaken in specific diagnostic categories where they were given, which were combined to broader categories where feasible and reviewed by the lead morphologist Dr Burthem to ensure the coding encompassed the essential elements of each participant's comments. For example in Case 4 responses such as follicular lymphoma and mantle cell lymphoma were combined as lymphoid neoplasms. Following this process the common outcomes were modified or refined to reduce the effect of random answers and to ensure the key diagnostic or interpretative information that the participant had

reported was maintained for analysis. Once agreed the process of coding all free text for that case was then undertaken. The total number of options depended upon the complexity of the case. Finally where interpretation of the free text was problematic those comments were again reviewed by myself, jointly with Dr Burthem. The final diagnostic categories and the number of participants are shown in the results section for each case.

Although participants were encouraged to suggest an overall summary of their morphological comments they were not required to do so and were not penalised for omitting this. As the free text data field was not mandatory for those participants where no conclusion was completed or where distillation of the free text to a diagnostic conclusion was not possible, the morphological diagnosis was coded as “not available”. This allowed the other sections of the data (priority of features and MCQ) to be analysed independently.

The free text questions for each case and the coded outcomes are given individually in Chapter 4 against the appropriate case.

3.3 Limitations of data analysis

The UK NEQAS(H) DM scheme for CPD is unique, with no national or international equivalent of similar scale and no precedent for how to approach the data analysis.

3.3.1 Professional skill level for morphology reporting of participants

The UK NEQAS(H) DM scheme had been developed to provide educational support for professionals who report blood cell morphology. The “carrot” for participation being that the scheme was accredited by the IBMS for CPD and that it also guaranteed anonymity of participants. UK NEQAS(H), therefore, had no information stored on the skill level of participants. During the data analysis of the first case selected for this thesis (scheme 0902DM), the fact that the level of morphology experience was unknown became a concern when analysing participant responses. It might be assumed that those providing the more correct reports were those with more experience and higher skill level but the DM scheme operated with the morphology experience of participants simply not known. Whilst this had always been a factor for

consideration when producing cases it now presented a problem for analysis of participant performance. After discussion and agreement via the morphology SAG the problem was presented during a feedback session to participants at the annual UK NEQAS(H) symposium and delegates were asked if they would support the incorporation of experience levels into their individual reports, their response was an overwhelming majority in favour. Permission was then sought and obtained from the Morphology SAG, prior to the release of Case 5 (Scheme 1204DM), to tailor the MCQ for that specific case in order to obtain information about the perceived skill level of participants. As participant data cannot be individually identified between cases the skill level question relates solely to Case 5. Participants were asked to select from a list of options the one which they felt most accurately represented their professional status including an option to decline the information, results are shown in section 4.5.3.

3.3.2 Lack of trial conditions

Completion of morphology cases by DM scheme participants was not completed under conditions normally associated with research studies, the data was extracted retrospectively. Participants had not completed cases in order to take part in a study but to complete educational exercises, during feedback sessions at the annual symposia the team had actively encouraged participants to share their knowledge of morphology for training purposes.

Some participants may complete cases simply to gain the CPD points without much care for what they submit and others do so because their workplace requires it. It must be recognised, therefore, that some feature selections must be random or made with little professional consideration. It is not possible to totally remove this effect, however where consensus agreement those selections are considered in the data analysis but where numbers for features selected are low (less than five individuals) the data was recorded, coded and considered but excluded from statistics.

3.3.3 Bias at coding of data

The free text interpretative morphological diagnosis given by participants reflected not only their summary report of the actual morphological features of the case but required them to interpret those features they had selected. For some cases it should have been possible to get an exact morphological diagnosis from the image, *e.g.* Case 2: Pelger–Huët anomaly as the key feature is a diagnostic one. This made coding a relatively simple process, separating those that specifically selected Pelger neutrophils from those who did not. In other cases *e.g.* Case 4: ATLL, a full diagnosis would have required other results including leucocyte immunophenotyping, however, the less experienced morphologist should recognise that the abnormal lymphoid cells were neoplastic, not simply reactive, this was key to providing a correct overall interpretation. The number of different selections for features associated with reactive or neoplastic lymphoid cells is broad so diagnostic options were agreed prior to coding and then revised further in light of actual participant reports to reduce the bias of expectation. The aim was to group free text reports by how close they were to an ideal report and also how close they came to conveying the correct diagnosis in that report.

Chapter Four: Results with interpretative comment

The number of participants who completed the selected cases for examination ranged from 732 to 1,108 individuals (median 878, Table, 3.1).

Images representing the cases studied are shown for each case in the appropriate results section. Each gives a limited stitched field section at low power on the left (representing between four to six fields) and a single field section at high power on the right which depicts the key morphological features (original stitched images presented to participants were 60 to 120 fields taken with a x63 oil immersion objective). The limited clinical or technical information originally presented to participants with the DM images are reprinted directly from the UK NEQAS(H) scheme shown beneath each image.

4.1 Single morphological feature Case 1: viral infection (EBV causing glandular fever)

Typical reactive polymorphic T lymphocytes found in a patient with a viral infection (EBV) against an otherwise normal cellular background.

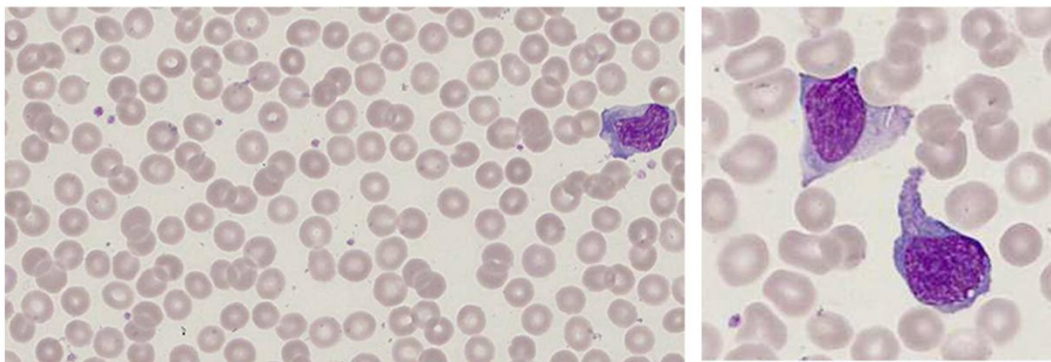


Figure 4.1. Case 1: viral infection (EBV causing glandular fever).

Original DM scheme information:

“For this film we have not provided any blood count parameters. This blood film was prepared from a sample sent from the Accident and Emergency Department marked: skin rash, enlarged lymph glands, and palpable spleen. ?leukaemia or lymphoma. ” Narrated by J Burthem and M Brereton”.

Originally distributed by UK NEQAS(H) as DM Scheme 0902DM. The key morphological feature was the atypical lymphocytes; they have a varied appearance but are generally larger and with a more square outline than seen when lymphocytes are in a normal

state. They have large amounts of basophilic cytoplasm that spreads outwards towards the surrounding red cells and a nucleus, which although large, appears mature (Figure 4.1). Importantly the abnormal lymphocytes are situated against an essentially normal background of other blood cells.

When reviewing the image participants should have considered any alternative explanations for the abnormal lymphoid cells, which lacked features associated with neoplastic or primitive cells, and they should also have noted that the other cells (red, white and platelets) were normal in appearance. Building this picture should have directed participants to a morphological diagnosis of a viral infection. In a real laboratory situation staff would usually perform a simple rapid diagnostic test (RDT) for the heterophile antibodies associated with glandular fever, to confirm their suspicions and direct their subsequent actions. A smaller number of laboratories may also have access to immunophenotyping and may be able to run simple lymphocyte markers to establish the nature of abnormal lymphocyte populations. When reporting on the DM case participants did not have access to these supplementary tests and endeavoured to reach their conclusion on morphology alone, this lack of supplementary information may then be reflected in their free text comments.

To obtain the correct morphological diagnosis in this case the participant needed to successfully demonstrate that they were able to:

1. detect the atypical lymphocytes.
2. correctly identify the atypical lymphocytes as reactive rather than neoplastic.
3. prioritise the atypical lymphocytes as rank 1 (the most important morphological finding in the image).
4. interpret correctly and conclude that the morphology was suggestive of a viral infection (lymphoid cells classed as the sole abnormality with or without recording the additional presence of normal morphology in other cell lines).
5. recognise that the addition of a simple screening RDT test (for glandular fever) should be the immediate action.

Number of participants reported as completing this Case = 732. Participant data removed due to incomplete, ruined or corrupt data sets = 17. Total remaining for detailed analysis = 714.

4.1.1 Morphological feature selection

Table 4.1 Variety of morphological features selected by cell lineage

	Red cell features	White cell features	Platelet features	Total
Morphological feature ranked as 1 (Top priority) by participants	6	25	3	34 of 74 options
Total number of features selected by all participants (all ranks 1 to 5 inclusive)	276	2,160	352	2,788

- Feature selections: 64 different morphology features were selected by at least one participant in their top 5 from the 74 options available.
- There were varied options for abnormal lymphocyte morphology which accounts for the high number of different white cell features selected (Appendix D).

Despite the overall variety of features selected abnormal lymphocyte features were selected as the top rank priority by 696 (97.4%) participants. Of those, 230 (one third), had incorrect selections *e.g.* blast cells or neoplastic.

4.1.2 Free text diagnosis question

“What is your preferred diagnosis based on the blood film appearances, you are allowed 3 options but please put your preferred option first”.

The preferred diagnosis was coded in to the groups shown in Table 4.2

Table 4.2 Summary of morphological diagnosis grouped by overall conclusion reported

Morphological diagnostic summary	Total	%
1/ Correct: Reactive lymphocytes (viral infection)	459	64.0
2/ Neoplastic lymphocytes (leukaemia, lymphoma, lymphoproliferative)	137	19.1
3/ Reactive or Neoplastic (combined, or neoplastic) but would complete RDT for glandular fever.	52	7.4
4/ No diagnostic summary or stating unable to make a morphological diagnosis	66	9.2

It was encouraging that most participants felt able to offer a morphological diagnostic summary and that, importantly, the majority were correct. The immediate question raised by the data being that if an incorrect diagnosis was made what was the nature of the misinterpretation? Examining the feature selections the key was correctly identifying the atypical lymphoid cells as reactive and not as blast cells; 106 participants (15 %) stated blast cells or neoplastic lymphoid cells as their top priority. An important factor in issuing a correct report being that 32.2% selected blast or neoplastic cells amongst their feature selections but only 26.5% included these in there summaries so not every participant who selected blasts or neoplastic cells went on to interpret them as a neoplastic disorder. Whilst the selection of blast cells was incorrect, it is not acceptable to report blast cells as a feature and then fail to account for them in an interpretative report; this could cause concern for the clinical team.

Feature selection for this case was also about recognising that the other cell types are normal in appearance and in normal quantity and so not selecting features such as thrombocytopenia which was an incorrect finding. It can be shown that the selection of incorrect features was then used by those participants to support the incorrect interpretation of a neoplastic condition (Figure 4.3 panel A). The building of an

incorrect report by over-reporting of absent features to support an incorrect assumption about the priority features seen will be discussed.

It is not known whether or how the clinical details given with this case (Figure 4.1) influenced the participants thinking. The clinical team recognised an unwell patient with enlarged lymph nodes, and their query over whether leukaemia or lymphoma was present should have made the film report an urgent matter in the laboratory. Whilst such clinical information is very useful in guiding the morphologist to consider possible diagnosis it must be the actual morphological features seen that are used to produce the report.

4.1.3 Action selected

The multiple choice question: “This blood film has arrived in the laboratory at the end of the day, what would you do?” (Participants were asked to select their preferred option see Table 4.3).

Anticipated action: The abnormal lymphocytes and the other normal morphology features, along with the age of the patient, should have led participants to consider the possibility of a viral infection (glandular fever being the most likely) and thus consider performing the simple and quick kit test. This would have enabled an immediate diagnostic cause for the abnormal cells to be available same working day, some participants may also consider immunophenotyping if their laboratory offers that service in its repertoire.

Table 4.3 MCQ: Participants preferred action

Action selected	Total	%
A/ Request a further test or tests	277	36.8
B/ Refer to a more experienced colleague or medic	220	30.8
C/ Issue a report describing the appearances and your diagnostic impression	111	15.5
D/ Describe appearances and ask for a repeat sample after an interval	29	4.0
E/ Issue a report and contact the referring clinician urgently	77	10.8

In private laboratories the cost of additional tests may prohibit staff from considering option A. It is not known how many participants this might apply to.

Inexperienced morphologists may refer a suspected viral infection to a colleague (action B), however it would be normal practice to report such a film directly, so the question of whether the action is linked, not only to experience, but to the interpretation of the morphological features seen is key to deciding whether this action is acceptable as summarised in Table 4.4 below.

Table 4.4: Actions taken for each morphological diagnosis summary group

Diagnostic summary	Action A (Further test)	Action B (Refer)	Action C (Issue report)	Action D (repeat)	Action E (urgent contact)
1/ Correct	55.5%	11.1%	21.1%	5.4%	6.8%
Viral Infection					
2/ Incorrect	5.8%	67.9%	4.4%	<1%	21.1%
Neoplastic					
3/ Incorrect	19.2%	55.8%	9.6%	0.0%	15.4%
Both options					
4/ None or unable to conclude	9.1%	69.7%	3.0%	4.5%	12.1%

Actions selected are shown as a % of participants who selected the diagnostic summary in column 1.

Action E, urgent contact with the referring clinician, is only of value if the correct report (viral infection) is being relayed, if the report is a neoplastic condition the effect of that phone call could have serious implications and cause unnecessary further tests to be performed, along with possible distress to the patient.

Those considering a neoplastic cause for the atypical lymphoid cells were far more likely to refer the film to another colleague; this should have corrected any inappropriate comments or actions but might be considered a disappointing finding for an image of such a common condition.

Those not considering a viral diagnosis were also less likely to suggest the addition of an RDT for glandular fever which would have allowed them to adjust their report and actions appropriately and stopped them from communicating an incorrect conclusion.

4.1.4 Case 1: summary findings from data

Examination of this case showed that:

- A. It is essential to correctly identify a significant feature
- B. The identification of the feature (whether correct or incorrect) impacts on the reporting of other features (which may not be correct) and thus bias the emphasis of the final report.
- C. The interpretation of the features seen will influence the outcome action (even when a diagnostic summary is not reported).

This last finding is important, it can be generally and professionally assumed that BMS provide a predominantly technical report of the morphological features they have seen and refer to a clinical lead any findings that require clinical interpretation. These results suggest that it is actually their interpretation of the features they see that control the subsequent actions.

To retest these findings another case was chosen for detailed analysis which had also depicted a single abnormal feature against an essentially normal background. The case selected had an abnormality of the neutrophils rather than the lymphocytes and was an inherited abnormality rather than an acquired transient phenomenon.

As both Cases 1 and 2 represented one abnormality the data from Case 1 is presented in more depth along with that from Case 2 in Figure 4.3 given after the results below.

4.2 Single morphological feature Case 2: Pelger-Huët anomaly

Typical bi-lobed Pince-nez like nuclei of neutrophils with condensed chromatin. The neutrophil granulation and cellular appearance are normal and so not supportive of either a septic or dysplastic conclusion.

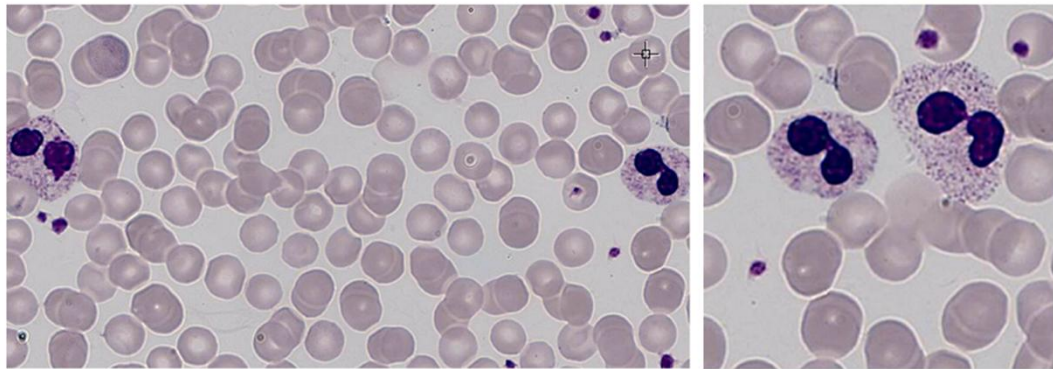


Figure 4.2. Case 2: inherited Pelger-Huët anomaly

Original DM scheme information: “This film was made following a routine blood screen prior to elective surgery. The blood film was prepared in response to ‘flagging alerts’ from the analyser. The full blood count indices were all within reference limits.” Narrated by M Brereton and J Burthem

Originally distributed by UK NEQAS(H) as DM Scheme 1206DM. Similarly to Case 1 the image of this blood film (Figure 4.2) contained one highly significant feature, the abnormal neutrophil of Pelger-Huët anomaly, in the morphological context of otherwise normal blood cells.

Unlike Case 1 which depicted a commonly seen viral infection (abnormal lymphocytes) Case 2 depicts a rare congenital condition (typified by the abnormal neutrophils), whilst far fewer participants would have seen a blood film of Pelger-Huët in their own laboratory it is a well described abnormality and examples are depicted in professional text books (Bain, 2005). It would be expected that this disorder is taught during training, the neutrophil is the most common leucocyte present in peripheral blood and changes in its appearance must be recognised. The morphological diagnosis carries no clinical implications for the patient, however, alternative potential explanations of the appearance needed to be considered by participants before reaching this conclusion. In particular the appearances had to be distinguished from the *pseudo*-Pelger neutrophil associated with myelodysplastic syndromes (MDS), a disorder which would

have significant clinical implications for the patient. *Pseudo*-Pelger cells found in MDS are often hypo-granular, found along with neutrophils with more nuclear lobes and are usually associated with abnormal features found in red cells or platelets. The true Pelger neutrophils also needed to be distinguished from the left shifted neutrophil associated with infection and sepsis, which would also have a potential significance for a pre-operative patient. Neutrophils with left shifted nuclear lobes (hypo-segmented) tend also to be more variable in appearance than true pelger cells and often have more prominent granulation due to increased myeloperoxidase activity.

To obtain the correct morphological diagnosis in this case the participant needed to be able to:

1. detect the abnormal neutrophils.
2. correctly identify the abnormal cells true Pelger neutrophils (as the sole abnormality with or without recording the additional presence of other normal cell lines).
3. prioritise Pelger cells as rank 1 (the most important morphological finding).
4. recognise that urgent action was not required as the condition has no clinical implications, but that the report should be brought to the attention of clinicians so that the correct interpretative report is made to the medical team.

Number of participants reported as completing the case = 1,108. Participant data removed due to incomplete, ruined or corrupt data sets = 80. Total completing all elements for detailed analysis = 1,028.

4.2.1 Morphological feature selection

Table 4.5 Variety of morphological features selected by cell lineage

	Red cell features	White cell features	Platelet features	Total
Morphological feature ranked as 1 (Top priority) by participants	9	14	4	27 of 74 options
Total number of features selected by all participants (all ranks 1 to 5 inclusive)	988	1,748	1,284	4,020

- 541 (52.6%) participants made less than 5 morphology feature selections (lowest number for any case studied).
- A total of 40 different features were selected by at least one participant from the 74 options available (lowest number for any case studied).
- Total number of morphology features in rank 1 was 27.
- Number of participants who selected abnormal neutrophils as their top feature = 861 (83.8%) of which 725 (70.5%) specifically chose Pelger cells as their top feature.

A morphological diagnosis was offered by 905 participants (88%) and most correctly reported Pelger-Huët anomaly (n = 584, 56.8%). However, two other clearly defined groups were apparent: MDS (n = 142, 13.8%) and infection (n = 54, 5.3%) see Table 4.3. Those reporting true Pelger-Huët also made the fewest selections (mean 3.6 from possible 5) which is appropriate as the other cells were essentially normal. This statistically differed from those selecting MDS (4.3 of 5, p = 6.7 SEM-6) where additional selections around red cell changes were selected, and from “reactive” (4.0 of 5 p = 4.5 SEM-5) where increased selections of toxic granulation were also made.

4.2.2 Free text diagnosis question

“What is your preferred diagnosis based on the blood film appearances”

Table 4.6 Summary of morphological diagnosis grouped by overall conclusion reported and showing participants ranking Pelger neutrophils as rank 1

Diagnostic summary	Total	%	Ranked Pelger neutrophils at rank 1 (feature of top priority)
1/ Correct: Pelger-Huët anomaly	584	56.8	499 (85% of group A)
2/ Myelodysplastic Syndrome	142	13.8	116 (82% of group B)
3/ Reactive or infection	54	5.3	8 (14.8% of group C)
4/ Other (malignant)	84	8.2	38 (45% of group D)
5/ Other (non-malignant)	41	4.0	7 (17% of group E)
6/ None	123	12.0	56 (45.5% of group F)

The abnormal neutrophils were noted by virtually all participants. Unlike Case 1, where participants reported the abnormal lymphocytes as either reactive or neoplastic; here there were three main outcomes for the abnormal neutrophils: Pelger-Huët, dysplastic or reactive so four categories, based on the selected features were created and scored so that they could be separated:

- A. Feature selection appropriate for a diagnosis of true pelger cells (no other features or normal features selected).
- B. Feature selection appropriate to MDS including thrombocytopenia, tear drop poikilocytes, macrocytosis and hypogranular neutrophils with *pseudo*-Pelger neutrophils. None of these additional selections were correct.

C. Feature sections suggesting a left shifted or reactive population of normal neutrophils (*e.g.* toxic granulation of neutrophils, thrombocytosis). None of these additional features were correct.

D. Feature selections suggesting other causes for the appearances (*e.g.* neoplastic lymphoid cells, microcytosis). Incorrect features resulting in an incorrect report.

Scores given to distinguish morphological features per participant:

1. Diagnostic (Pelger neutrophils identified): required observation +0
2. No selection made (appropriate as film otherwise was normal) +0
3. Non-specific (codes associated with normal film also appropriate) +0
4. Reactive (features associated with reaction amongst myeloid cells): incorrect +1
5. Dysplastic (features that imply a diagnosis of myelodysplasia): incorrect -3
6. Features not in any category above but also not present on film: incorrect -2

Although fewer morphological features were selected than for other cases studied ($n = 40$) this was still a wide selection. To facilitate analysis, these feature selections were grouped into three sets that reflected their pathological significance:

- Group 1 (assigned score = 0) were consistent with the correct diagnosis Pelger-Huët on a normal background.
- Group 2, choices indicative of a reactive state were assigned a score of +1 (*e.g.* toxic granulation, thrombocytosis).
- Group 3, choices most consistent with possible myelodysplasia were assigned a score of -1 (*e.g.* hypogranular neutrophils, thrombocytopenia, tear drop poikilocytes).

Thus a score of 0 indicated a morphological syndrome entirely indicative of Pelger-Huët anomaly with no other abnormal features. Deviation from this with positive bias indicated features suggestive of a reactive blood film, while a negative bias indicated features most consistent with myelodysplasia. Using this system, participants who chose a diagnosis of Pelger-Huët selected features whose morphological score was closest to 0 (mean = 0.3, SD = 1.1). Where MDS or infection were chosen, both had an additional bias. For MDS this was not significantly different from the features selected by the Pelger-Huët group (mean = 0.5, SD = 1.2 $p > 0.05$); however for those of the

group choosing infection the positive bias was highly significant (mean = 1.4, SD = 1.3 p = 1.2 SEM-12). The different scores between the “infection group” and the Pelger or MDS groups might arise solely because of a misclassification of Pelger cells (scored 0) as left shifted neutrophils (scored +1) by these participants.

To address this, analysis was repeated with left shifted neutrophils assigned a score of 0 (equivalent to Pelger neutrophils). This reduced the positive score of the infection group, but the score difference compared with the Pelger group remained significant (mean 0.7 +/- 1.0, p = 0.007) suggesting that additional features were being sought to support the assumption of infection. This data is shown in Figure 4.3 panel B.

4.2.3 Action selected

Multiple choice question: Which one of the following options best describes how you would manage the review of this case?

Anticipated answer: As a routine pre-operative case the participants should have recognised the congenital abnormality and otherwise normal blood cells and selected option “A” to report the film and then refer non-urgently for clinical comment.

Table 4.7 MCQ: Participants preferred action

Action selected	Total	%
A/ Film for medical comment/referral (non-urgent)	619	60.1
B/ Requires referral of film to haematologist (urgent).	188	18.3
C/ Report film appearances; no further action required.	201	19.5
D/ Urgent contact with referring clinical needed.	16	1.6
E/ No film report needed*	5	0.5

*Those selecting action E: no film report needed, did not offer a diagnostic summary and had not selected Pelger neutrophils amongst their morphological feature selections. They, therefore, had regarded the case as “normal”.

Although correctly reporting the findings of Pelger-Huët is the aim, not referring the film (option C) misses the opportunity for a clinical colleague to add valuable interpretation and advice to the medical team who may not be familiar with this condition or how to discuss the diagnosis with the patient.

As seen for Case 1, urgent referral to a clinical haematologist is unnecessary, could cause undue alarm and take the clinician away from more important matters. It might be acceptable if the participant is inexperienced but might imply the participant has misinterpreted the findings as MDS. The relationship between the participants' actions and their diagnostic summary is presented in the following table 4.8.

Table 4.8 Actions taken for each morphological diagnosis summary group

Diagnostic summary	Action A (Refer: Non-urgent)	Action B (Refer: Urgent)	Action C (Report only)	Action D (Urgent contact)
1/ Correct Pelger-Huët	67.6%	8.6%	23.3%	0.3%
2/ Incorrect MDS	54.2%	42.3%	2.1%	1.4%
3/ Incorrect reactive/sepsis	29.6%	16.6%	46.3%	7.4%
4/ Incorrect other (malignant)	48.8%	46.4%	1.2%	3.6%
5/ Incorrect other (non-malignant)	43.9%	19.5%	26.8%	9.8%
6/ No diagnostic summary	58.5%	17.9%	19.5%	0.8%

Actions selected are shown as a % of participants who selected a diagnostic summary as coded in column 1.

Action B; urgent referral due to incorrect classification of the main abnormal feature may cause unnecessary disruption to the clinical lead but should allow the report to be corrected before release, however, if the report had been issued then the patient could be caused unnecessary distress. Also of concern is the release of incorrect reports due to misclassification of the Pelger neutrophils as a reactive feature (action C). This incorrect action not only misses the opportunity to provide a correct diagnosis but may result in either an unnecessary treatment for a sepsis that doesn't exist or a delay in a planned procedure.

4.2.4 Case 2: summary findings from data

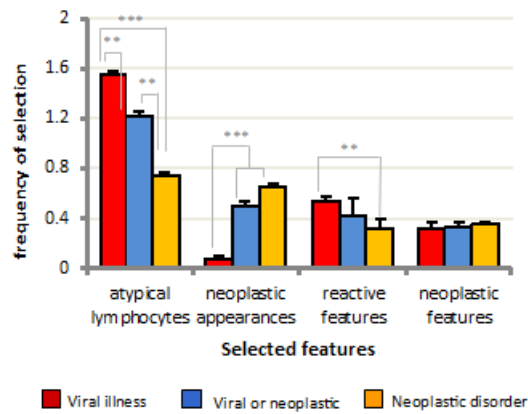
An examination of data from Case 2 showed that:

- Selection of this case for examination was shown to have been a fair test. Despite knowing that participants might not have seen Pelger-Huët in their own laboratory the correct diagnostic selection was high and most participants were correct.
- Neutrophil features (Pelger or left shift) were consistently given the highest priority, irrespective of diagnosis, *i.e.* initial assessment of the case was correctly carried out.
- Those reaching the correct actual diagnosis made the fewest selections of morphological features *i.e.* consistent with diagnosis and did not over report.
- The morphological diagnosis reached by participants depended almost exclusively on their interpretation of neutrophil morphology, although where infection was diagnosed there was a tendency to report supporting features that were either not there or not present sufficiently to justify reporting (*i.e.* the neutrophils did not have a significant level of toxic granulation).
- The actions taken by participants were strongly influenced by the interpretation of this single feature.

The data summary is consistent with that found for Case 1. Outcome data from these cases were then compared. For this analysis the actions for Case 2 were assigned scores (1 to 5 respectively). Thus those reports considered most clinically significant would receive the lowest overall score. When applied to the three groups those diagnosing MDS chose responses that were significantly different from the other groups and reflected the highest urgency (mean = 2.55 SD = 0.52, $p = 1 \text{ SEM-25}$), whilst the finding of reactive or Pelger had lower urgency scores which were not significantly different (Pelger-Huët: mean = 3.14 SD = 0.59, infection: mean = 3.14 SD = 0.96).

The data for the single feature cases (1 and 2) are further explored in the following Figure 4.3 (Brereton, De la Salle, Ardern, Hyde, & Burthem, 2015).

Panel A: Case 1 (viral infection: EBV)



Panel B: Case 2 (Pelger-Huët anomaly)

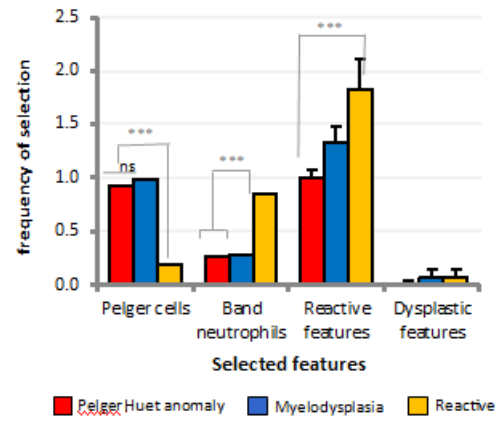
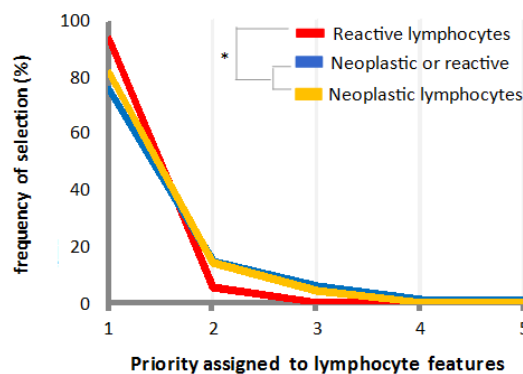


Figure 4.3 Panels A and B: Morphology features selected by participants for each case according to the interpretative diagnosis made. Bars represent the mean number of selections for the indicated feature or feature group (for groups, variability is indicated by bars representing SEM). Significant differences of selection frequency are indicated on the panels (Chi-square test). Statistical differences between cases are indicated on figure (ANOVA test). $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***

Panel C: Case 1 (viral infection: EBV)



Panel D: Case 2 (Pelger-Huët anomaly).

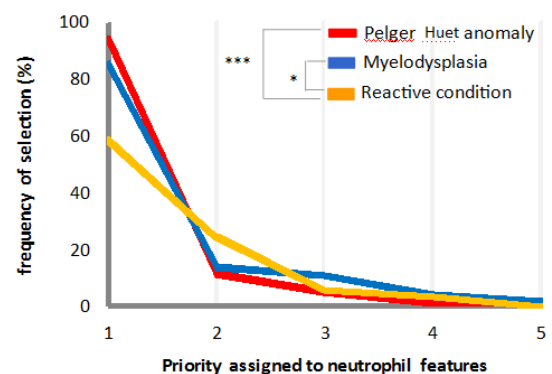
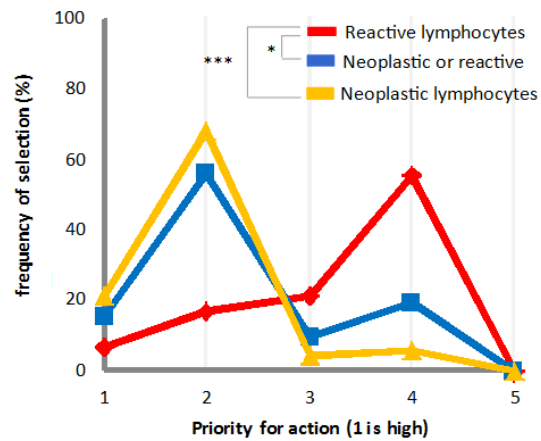


Figure 4.3: Panels C and D: Priority score for first selected feature from a morphological group shown according to the diagnosis made (1 is the highest priority and significant differences are indicated on the figure (Mann Whitney test)).

Figure 4.3 Analysis of participant responses from the 2 cases with a single morphological feature.

Panel E: Case 1 (viral infection: EBV)



Panel F: Case 2 (Pelger-Huët anomaly)

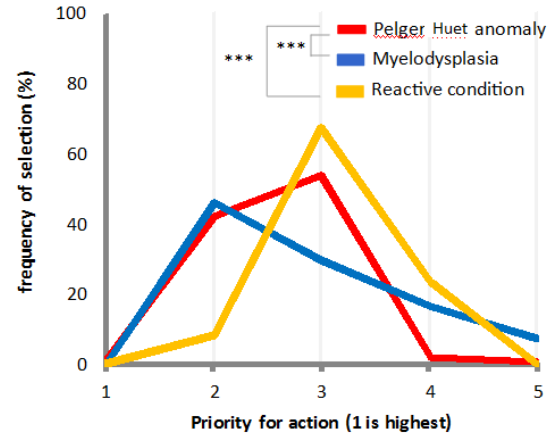


Figure 4.3 Panels E and F: Priority for action ascribed to case according to diagnosis (1 is highest priority).

Figure 4.3 Analysis of participant responses from the 2 cases with a single morphological feature. Significance is indicated as follows: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.

4.3 Complex morphology Case 3: Microangiopathic haemolysis and viral infection

Microangiopathic haemolysis showing features of thrombocytopenia, keratocytes, fragmentation and polychromasia. Reactive lymphocytes are seen in both panels.

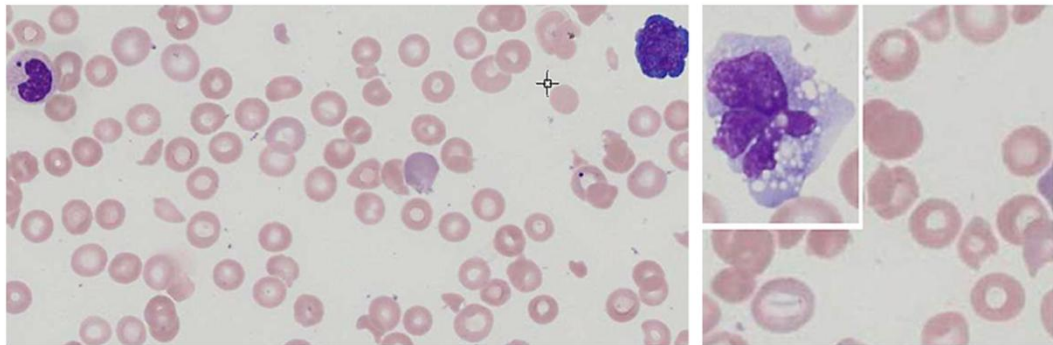


Figure 4.4 Case 3: Microangiopathic haemolysis with reactive lymphocytes of a viral infection

Original DM scheme information: "This image represents the blood film from a non-pregnant 23 year old female attending an evening clinic. An earlier blood count carried out by her General Practitioner had shown results within reference range for all parameters, however on the present occasion the platelet count was found to be $10 \times 10^9/l$. No clot was present in the sample. An urgent blood film was performed and the digital image is from part of this film" Narrated by M Brereton and J Burthem

Originally distributed by UK NEQAS(H) DM scheme as 0903DM. Actual diagnosis of thrombotic thrombocytopenic purpura (TTP) causing a microangiopathic haemolysis with human immunodeficiency viral (HIV) infection.

Cases 1 and 2 had depicted a single morphological abnormality, yet in a real reporting situation professionals are also expected to be able to identify and interpret the features found in complex clinical conditions. Case 3 depicts two different but related conditions, the first being the patient's blood response to a viral infection (new HIV infection). The second, an acute haemolytic process triggered inappropriately by the immune system in response to the treatment of that infection. The morphology shows a high number of large atypical lymphocytes caused by the immune response to a viral infection, coincident with the striking abnormal red cell morphology caused by the mechanical structural cell damage seen in microangiopathic haemolysis and associated thrombocytopenia (shown in Figure 4.4).

iatrogenic conditions are a rare but a well recognised part of medicine, so a morphologist should be alert to the possibility that certain combinations of abnormal morphology may appear together.

Basic diagnosis in this case required the participants to be able to:

1. identify the thrombocytopenia.
2. correctly classify and rank the order of red cell features.
3. examine all cell lines (red cells, white cells and platelets) in order to identify the abnormal lymphocytes having recognised the haemolytic process.
4. recognise that urgent action is required.

The questions raised by this case are complex: can participants process such a complicated set of morphological features to distill essential information for the clinical team and make a correct, concise report? If not where do participants fail?

Number completed according to scheme data sheet = 752. Participant responses removed due to incomplete or corrupt data sets = 6. Total remaining for analysis = 747.

4.3.1 Morphological feature selection

Table 4.9 Variety of morphological features selected by cell lineage

	Red cell features	White cell features	Platelet features	Total
Morphological feature ranked as 1 (Top priority) by all participants.	13	9	1	23 of 74 options
Total number of features selected by all participants (all ranks 1 to 5 inclusive)	2,279	616	721	3,616

A total of 63 different morphological features were selected by at least one participant from the 74 options available.

- The number of features ranked 1 (most important) by participants was lower than for any other case studied.
- Case 3 had been distributed by the DM scheme to participants directly following Case 1 as presented in this thesis, and so had been completed by

largely the same participant base. With similar numbers completing both cases (747 for Case 3 and 714 for Case 1) it is interesting that the total number of features selected here (3616) is notably higher than for Case 1 (2788) and reflects the markedly abnormal morphology of Case 3.

Table 4.10 Top features selected by priority for each major cell lineage

Cell lineage	Feature selected	Total	Selected %	Ranked 1 %
Platelet	Thrombocytopenia	700	93.7	60.0
red cell	Fragmented red cells	672	90.0	30.3
white cell	Abnormal lymphocytes*	446	59.7	1.9

*Abnormal lymphocytes: There were several possible morphology feature options for participants to select Appendix D) which reflect abnormal and reactive lymphocytes so these have been combined.

4.3.2 Free text diagnosis question

“Suggest your preferred diagnosis based on your morphological observations and the supplied clinical details”.

The preferred diagnosis was coded in to the groups shown in Table 4.11.

Table 4.11 Summary of morphological diagnosis grouped by overall conclusion reported

Diagnostic summary	Total	%
1/ Correct: MAHA plus viral illness	125	16.7
2/ MAHA alone	385	51.5
3/ Haemolytic process: non-specific or other cause <i>e.g.</i> haemoglobinopathy	157	21.0
4/ None given or unable to give summary	80	10.7

- No participants selected only reactive lymphocyte features, or gave a diagnostic report that summarised only abnormal lymphoid cells. All participants selected at least one abnormal red cell feature amongst their five choices including those who chose not, or were unable, to give a morphological diagnosis.
- The experience level of those unable to correctly interpret their findings is not known (groups 3 and 4), however, virtually all participants ranked thrombocytopenia highly so in a real laboratory situation the film should have been referred allowing a more experienced morphologist to correct the report.
- Considering the complexity of this case it is not surprising that so many chose not to summarise their findings (group 4).

The immediate question raised by this data is why did so few include the abnormal lymphocytes in their summary report? (group 1). Over half of participants had selected abnormal lymphocytes amongst the features they reported yet this is not reflected in their summary reports. Arguably BMSs do not provide interpretive reports so have a limited skill set in this part of film reporting. The film would have been referred for clinical review so a clinical interpretation for the lymphocytes would have then been added. It must be considered, however, that having realised the significance of the

haemolytic process the atypical white cells were simply ignored as they did not fit in as part of the red cell haemolysis.

4.3.3 Action selected

Multiple choice question: How could you best describe your reaction to this film? Please select your preferred option.

Anticipated answer: The acute thrombocytopenia and presence of fragmented red cells and keratocytes indicate an acute mechanical haemolytic event, delay in communicating these findings could contribute to the death of the patient by delaying appropriate intervention; immediate action could have a positive impact on patient outcome.

Table 4.12 MCQ: Participants preferred action

Action selected	Total	%
A/ Emergency immediate action is required	694	92.3
B/ Urgent referral – action next working day	44	5.9
C/ Referral – requires further opinion, non-urgent	8	1.1
D/ Issues a routine report	1	

With virtually all participants taking appropriate action there was no requirement to compare actions against their summary reports.

4.3.4 Case 3: summary findings from data

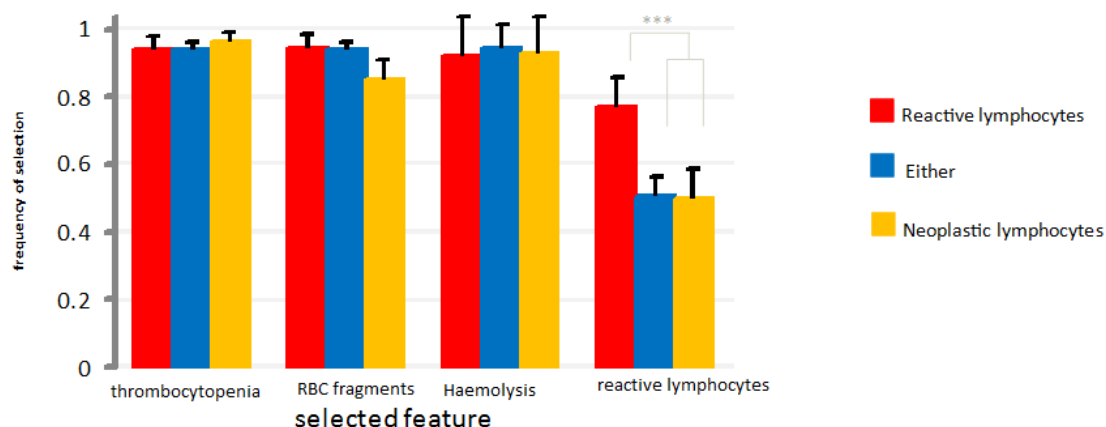
Examination of Case 3 showed:

- The high level of urgency given to this case with only 53 participants not understanding the need for immediate action.
- A high number prioritised thrombocytopenia and nearly everyone selected this as an important feature, explaining why the total number of features selected as top priority was low for this complex case.

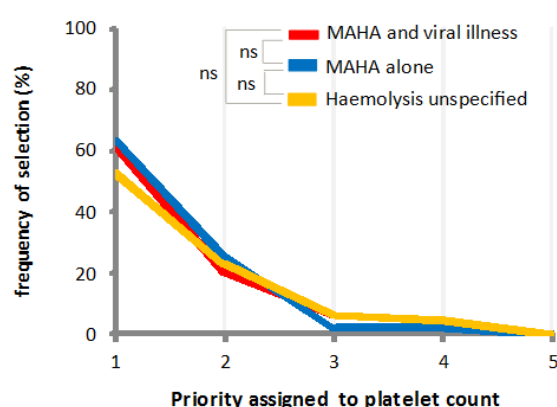
- In a laboratory situation the detection and communication of thrombocytopenia is clearly well recognised but the additional message concerning the specific nature of the haemolysis would have added value to the report. The data suggests the level of skill required for this is not as high.
- Despite the high recognition of the thrombocytopenia some participants either did not, or were unable to, include thrombocytopenia in their interpretation and thus did not reach a correct morphological diagnosis.
- Fragmented red cells were selected correctly by the vast majority of participants (90%) but again many either did not, or could not, interpret the importance of this feature in to their final morphological diagnosis (Table 4.11).
- Importantly, most participants selected both thrombocytopenia and abnormal red cells, but only 59.7% of participants selected abnormal lymphocytes as a feature (suggesting nearly 40% either missed them, did not consider them important, or did not examine the white cell morphology). Furthermore only 16.7% included the abnormal lymphocytes in their morphological summary.

The important question raised by this case data is why did the participants not comment on the abnormal white cells? The implication has to be that having found the haemolytic process they stopped the examination. This question is considered in the following Figure 4.5 panels A to E.

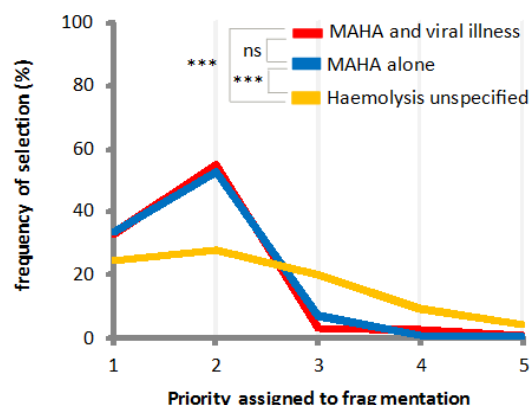
Figure 4.5 Panel A:



Panel A: Frequency of abnormal lymphocyte selection by diagnostic summary. Major morphological feature groups shown as the mean number of selections for the indicated feature. These features are divided according to diagnosis and variability are represented by error bars (SEM). Significant differences are indicated on the figure (Chi-square test).

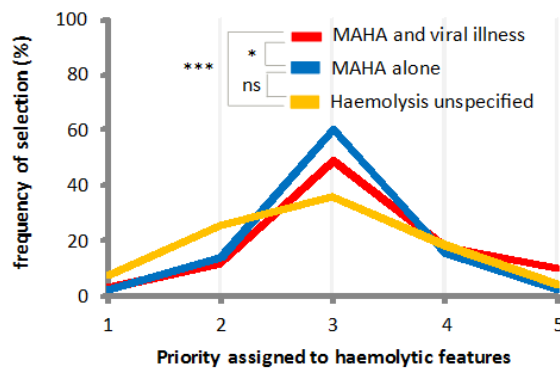


Panel B: Priority of thrombocytopenia

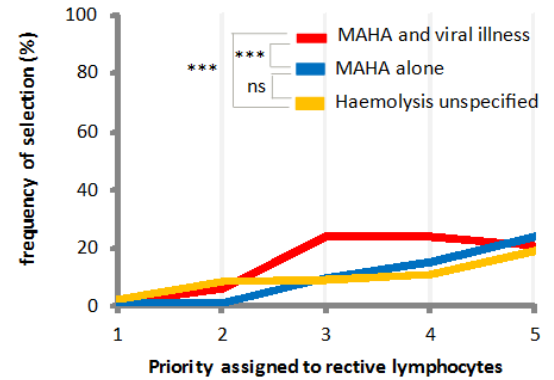


Panel C: Priority of fragmentation

Figure 4.5 Analysis of participant responses in a complex case with related morphological features. Case 3: Microangiopathic haemolytic anaemia associated with viral illness (acute HIV infection). Significance is indicated as follows: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.



Panel D: Priority of haemolytic features



Panel E: Priority of reactive lymphocytes

Figure 4.5 Panels B to E: Priority score given to the first selection of any feature from a feature group (1 is the highest priority), selections are divided according to the given diagnosis. Significant differences are indicated on the figure (Mann Whitney test). Figures represent each major morphological feature groups: platelets (A), fragmentation (B), haemolytic features (C), reactive lymphocytes (D).

Figure 4.5 Analysis of participant responses in a complex case with related morphological features. Case 3: Microangiopathic haemolytic anaemia associated with viral illness (acute HIV infection). Significance is indicated as follows: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.

4.4 Complex morphology Case 4: Haemoglobinopathy and acute leukaemia

A haemoglobinopathy showing frequent target cells, contracted red cells, polychromasia, folded boat-shaped red cells and nucleated red blood cells. There is also abnormal neutrophil morphology and primitive leucocyte precursors (blast cells) indicating acute myeloid leukaemia.

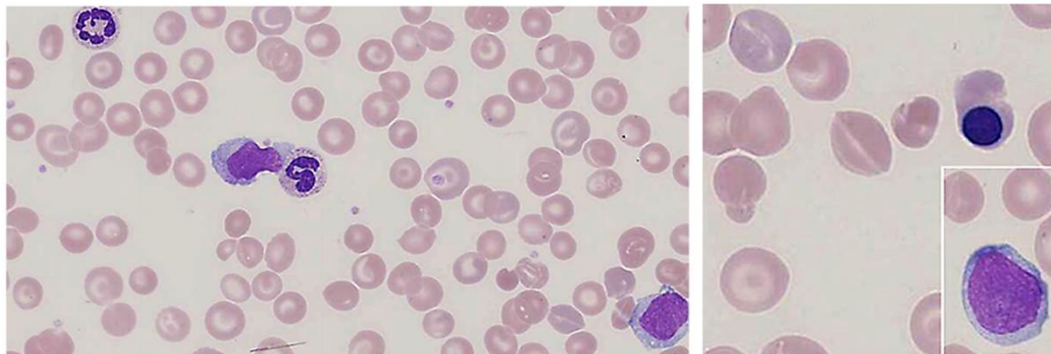


Figure 4.6 Case 4: Haemoglobin SC disease with acute myeloid leukaemia

Original DM scheme information: “A 72 year old man. This blood count was sent from a hospital clinic. The count shows Hb7.5g/dl, WBC $18.0 \times 10^9/l$, platelets $310 \times 10^9/l$. The image presented is from a blood film is made at the time. Narrated by M Brereton and J Burthem”.

Originally distributed by UK NEQAS(H) as DM Scheme 1201DM. Actual diagnosis of the inherited disorders haemoglobin sickle cell (Hb S) combined with haemoglobin C (Hb C) termed HbSC plus acquired acute myeloid leukaemia (AML).

In Case 3 the two separate sets of abnormal morphological features were derived from two clinical conditions that were pathologically linked. Arguably this is the most likely scenario found in the laboratory, examples being haemolytic anaemia found in patients being treated for chronic lymphocytic leukaemia, lymphoproliferative disorders arising in heavily immunosuppressed patients post renal transplant, or thrombotic thrombocytopenia developing in patients post allogeneic or non-related donor stem cell transplant. The morphologist, however, must report what they see, not just what they expect to see, they must be aware of what is acceptable in a clinical

condition and what is not, they must be alert to scenarios that are rare or unique and they must never assume that an unexpected finding can be ignored.

Case 4 depicts a rare (possibly unique) and complicated blood film with many notable and reportable features (Figure 4.6). In Case 3 the numerous red cell features appeared to distract some participants from providing a full report, in Case 4 there are also multiple red cell abnormalities. In this instance, the key erythroid feature is the target cell, as found in a variety of haemoglobinopathies. Other causes for the target cells must be considered and it important that participants consider the size of the target cells and the other abnormal features they are associated with. If the target cells were those seen in liver disease then one would expect them to be larger than those usually found in haemoglobinopathies and a larger number of stomatocytes might also be present. Additionally there is a thrombocytopenia, which might also be seen in liver disease, but is associated with the presence of a secondary clinical condition which is unrelated to the haemoglobinopathy. In this case, however, that secondary condition is the onset of acute myeloid leukaemia, for which blast cells are the key morphological finding. This case demonstrates an acute pathological condition evolving separately over a pre-existing chronic condition.

Therefore, Case 4 requires the participants to be able to:

1. find abnormalities in all cell lines.
2. correctly identify those abnormalities
3. correctly associate abnormalities with their clinical cause.
4. correctly prioritise the abnormalities found and stratify according to the underlying pathology.
5. recognise that blast cells are not an expected or acceptable feature of a haemoglobinopathy.

Participants must have the knowledge and skill to identify features and be able to separate those features which they might expect to see from those that are not associated with condition.

Number of participants reported as completing the case = 1,011. Participant data removed due to incomplete, ruined or corrupt data sets = 68. Total completing all elements for data examination = 948.

4.4.1 Morphological feature selection

Table 4.13 Variety of morphological features selected by cell lineage

	Red cell features	White cell features	Platelet features	Total
Morphological feature ranked as 1 Top priority by participants.	15	23	3	42 of 74 options
Total number of features selected by all participants (all ranks 1 to 5 inclusive)	2,894	1,434	318	4,646

Total of 64 features selected from the 74 options available. A notably high variety of feature selections were chosen as the top priority.

Table 4.14 Top features selected by priority for each major cell lineage

Cell lineage	Feature selected	Total	Selected %	Rank 1 %
Platelet	Macrocytic platelets	225	23.7	0.5
red cell	Target cells	811	85.5	23.8
white cell	Neoplastic Leucocytes*	529	55.8	42.7

*Neoplastic leucocytes is combined from participant selections recognising myeloblasts, lymphoblasts or neoplastic abnormal leucocytes.

Whilst the majority of participants recognised the presence of red cell abnormalities far fewer went on to select features of the abnormal white cells (Table 4.14), however, if they did participants ranked their importance above that of the abnormal red cells (Table 4.13).

4.4.2 Free text diagnosis question

“Suggest your preferred diagnosis based on your morphological observations and the supplied clinical details.”

Interpretation and subsequent coding of the diagnostic comments was complicated, despite more than half of participants selecting both red cell and white cell abnormal features in their five choices only a quarter considered both when summarising their report.

- Diagnostic summary considered both red cell and white cell abnormalities: 237 = 25.0%
- Red cell conclusion only: 283 = 29.8%
- White cell conclusion only: 264 = 27.8%
- No diagnostic summary or stated would be unable to interpret. 164. = 17.3%.

As the urgent clinical finding is the presence of an acute leukaemia the morphological diagnostic summaries were grouped as to whether participants actually reported acute leukaemia.

The preferred diagnosis was coded in to the groups shown in the following table.

Table 4.15 Summary of morphological diagnosis grouped by overall conclusion reported

Diagnostic summary	Total	%
1/ Correct: Acute leukaemia and a haemoglobinopathy	84	8.9
2/ AML but either no red cell conclusion or an incorrect red cell conclusion	121	12.7
3/ Other neoplastic causes for the abnormal white cells (<i>e.g.</i> lymphoma)	232	24.5
4/ Sepsis or reactive (non-neoplasm)	227	23.9
5/ No abnormal white cells reported – Red cell only diagnosis made	283	29.8

- Those correctly interpreting their report as acute leukaemia = 205 (21.6%)
- Notably 24.5% made an incorrect interpretation of the blast cells but did suggest a neoplastic cause, whilst this is not correct it would have ensured further review.

- Those completing feature selections and answering the action MCQ but either not entering a free text diagnosis or stating that they were unable to make a morphological diagnosis was, not surprisingly, high compared to Cases 1, 2 and 3 $n = 164$ (17.3%).

Examination of red cell comments:

- Of participants who reported the correct morphological summary of Haemoglobinopathy (281 = 29.6%) most either failed to incorporate abnormal leucocytes in to their summary or reported an incorrect white cell conclusion which did not include a neoplastic element (197 = 20.8%).
- Other causes for the abnormal erythrocytes (*e.g.*, liver disease, hyposplenism, megaloblastic anaemia) were suggested by 300 participants (31.6%) who did not consider a haemoglobinopathy.

4.4.3 Action selected

Multiple choice question: Based on the information you have and your interpretation of the film what is the most appropriate course of action? Please select your preferred option.

Anticipated answer: Based on the unexpected finding of myeloblasts the film should have been referred immediately: the patient requires urgent clinical review.

Table 4.16 MCQ: Participants preferred action

Action selected	Total	%	*Neoplastic ranked at 1 (top priority) %
A/ Refer immediately- Patient may need to be seen urgently	573	60.0	55.8
B/ Report directly to medic within 24 hours – may need review next working day.	208	21.9	33.7
C/ Important – film findings need communication to clinician, non-urgent.	120	12.8	11.7
D/ A routine report is sufficient as patient is under haematological review	44	4.6	6.8
E/ No report required, this is a known chronic condition	3	0.3	0

*Neoplastic ranked at 1: Percentage of participants who reported case as neoplastic against the action category they selected.

The majority of participants would have referred this film and so the acute leukaemia would, at some point, have been recognised, but most were referring the film for the abnormal red cell morphology. In a real situation the haemoglobinopathy would probably have been already known so the case may not have been referred. The participants who did not select abnormal white cells referred the film with a lower level of urgency.

4.4.4 Case 4: summary findings from data

- The red cell features caught the attention of participants, although the majority struggled to interpret them correctly. It should be noted that although HbSC is a well described haemoglobinopathy such patients are often monitored in specialist centres and so many participants may not see such blood films routinely.
- The lack of skill shown in interpreting the red cell features resulted in poor summary reports; most participants chose to refer the film for review.

- The abnormal white cells were “missed” by almost half of participants but where they were reported they were correctly given a high priority.
- Despite the high priority given to the abnormal white cells this did not automatically trigger an urgent communication of these findings or appear as part of the interpretive summary report.

These findings are shown in detail below in Figure 4.7 panels A to E.

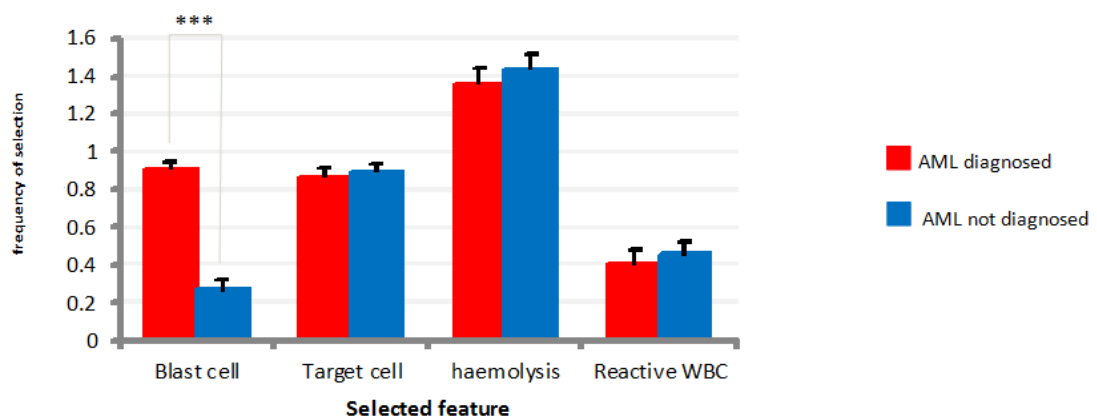
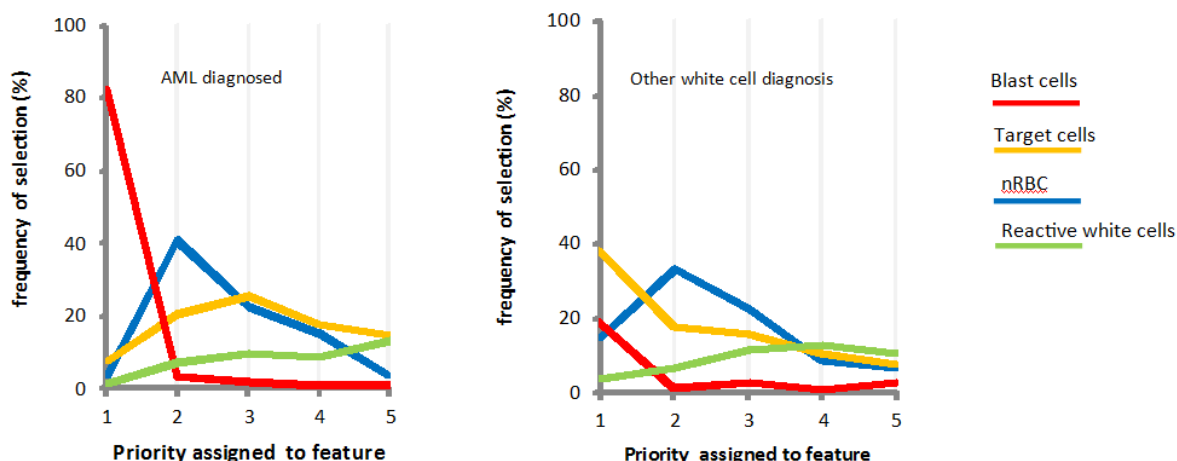
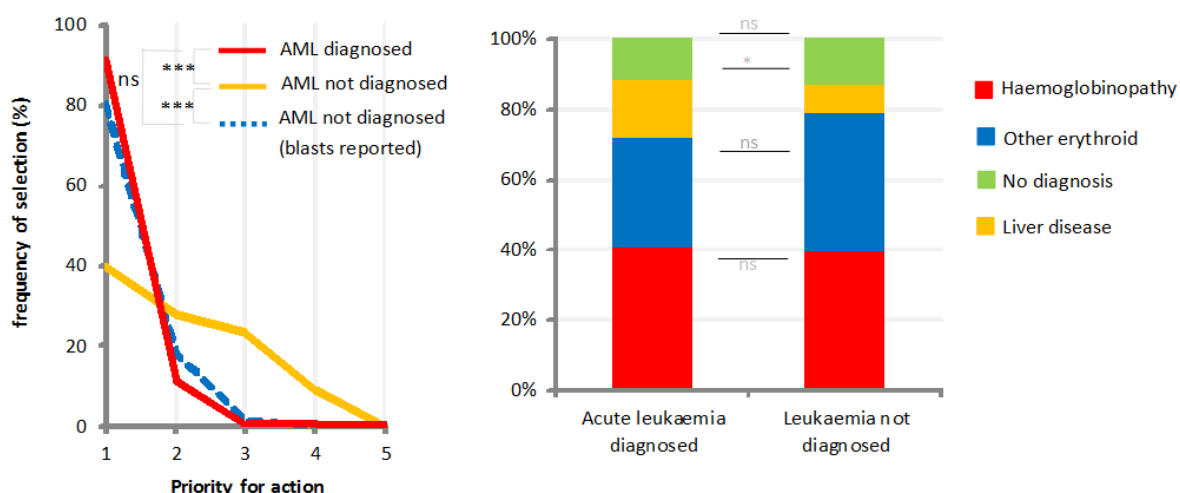


Figure 4.7 Panel A: Morphology features selected by participants reporting a diagnosis of AML. Major morphological feature groups represented according to whether a diagnosis of acute leukaemia was made, bars represent mean selection number with error bars (SEM) and significant differences are indicated on the figure (Chi-square test). Significance is indicated: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.



Panel B: Priority when AML Diagnosed Panel C: Priority for other WBC diagnosis
 Priority score given to the first selection of any feature each feature group (1 is the highest priority), panels reflect whether acute leukaemia was diagnosed (B) or not diagnosed (C).



Panel D: Action according to diagnosis Panel E: Red cell diagnosis against WBC diagnosis

Panel D: Priority for action taken according to diagnosis suggested (1 is highest priority). Priorities indicated reflect whether acute leukaemia was diagnosed, a third broken line (blue) represents the subset of participants who recorded the presence of blast cells but did not diagnose acute leukaemia in their summary report. **Panel E:** Red cell diagnoses offered according to whether acute leukaemia was diagnosed. Statistically significant differences are indicated on the figure (Mann Whitney test).

Figure 4.7 Case 4: Analysis of participant responses where morphological features reflected two separate unrelated pathological disorders (HbSC disease with AML). Significance is indicated as follows: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.

4.5 Complex morphology Case 5: skill levels known

Numerous bite cells and hemi-ghost red cells are distinctive features of oxidative haemolysis and are seen alongside other general features of haemolysis such as polychromasia and red cell damage. Also present are the abnormal primitive lymphoid cells of ATLL, the right panel shows a typical example with basophilic cytoplasm, lobulated and folded nuclei.

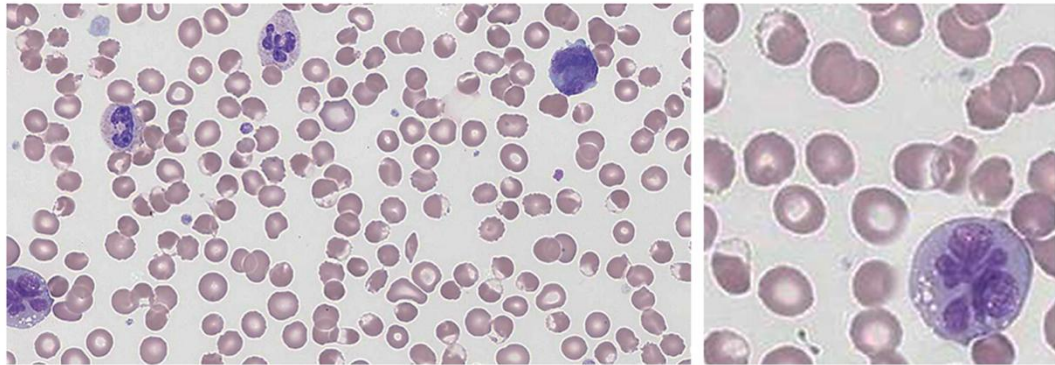


Figure 4.8 Case 5: oxidative haemolysis (G6PD deficiency) and adult T-cell leukaemia/lymphoma

Original DM scheme information: “A 60 year old male admitted as an emergency several days earlier and recently commenced on medical treatment now develops a change in clinical condition. Full blood count at the time of the sample was WBC $44 \times 10^9/L$, Hb 93 g/L, Platelets $72 \times 10^9/L$. “ Narrated by M Brereton and J Burthem”.

Originally distributed by UK NEQAS(H) as DM scheme 1204DM. Actual diagnosis of adult T-cell leukaemia/lymphoma (ATLL) on treatment, with therapy related acute oxidative haemolysis due to exacerbation of an undiagnosed deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD).

As in Case 3 this also shows a rare occurrence of two separate pathological disorders which are linked by treatment. In this instance the genetic deficiency of G6PD leads to an acute oxidative haemolytic process when exacerbated by clinical therapy given to treat the ATLL. The clinical team had no prior knowledge of the inherited G6PD deficiency as they had not tested for this. The subsequent acute haemolytic crisis was, therefore, avoidable but created a dangerous clinical situation for the patient. The key morphological features are those of atypical lymphoid cells with neoplastic features; notable folded, flower shaped nuclei against a background of markedly abnormal red

cells with distinctive “bite” shape and hemi-ghost cells which are distinctive features associated with acute oxidative haemolysis (Figure 4.5). This information requires urgent communication to the clinical team and the nature of the report should lead them to consider G6PD deficiency as the probable cause and intervention should be immediate. Failure to communicate these specific features of oxidative haemolysis could lead to delay in correct treatment and a catastrophic haemolytic destruction of the patient’s red cells.

For this case participants were asked to state their perceived skill level from options provided. The case required the participant be able to:

1. find abnormal features in all cell lines
2. correctly identify those abnormal features.
3. correctly prioritise the abnormal features found
4. to recognise that the specific features which identify the oxidative process as cause of the haemolysis require immediate communication, especially as at this point the haemoglobin concentration may not be sufficiently abnormal to cause undue concern for the clinical team.

Participants must have the knowledge and skill to identify features and be able to separate those features which they might expect to see in one abnormality from those that are not associated with the primary condition.

Number of participants completing this case = 789. Participant data removed due to incomplete or corrupt data sets = 17. Total remaining for analysis = 772.

4.5.1 Morphological feature selection

Table 4.17 Variety of morphological features selected by cell lineage

	Red cell features	White cell features	Platelet features	Total
Morphological feature ranked as 1 (Top priority) by participants	15	18	1	34 of 74 options
Total number of features selected by all participants (all ranks 1 to 5 inclusive)	1,647	1,409	450	3,506

Total of 63 features selected from the 74 options available. A high variety of feature selections were chosen as the top priority.

Table 4.18 Top features selected by priority for each major cell lineage

Feature selected	Total	selected	rank 1
		%	%
Ghost/Hemi ghost red cells	546	70.7	45.2
Thrombocytopenia	387	50.1	2.3
Neoplastic Lymphocytes*	270	35.0	13.6

*Neoplastic lymphocytes is combined from participant selections for the multiple options which indicate neoplastic lymphoid features.

- A high proportion recognised the key red cell destruction feature correctly.
- Neoplastic lymphoid features were poorly categorised.

4.5.2 Free text diagnosis question

“Suggest the diagnosis or diagnoses that, in your view, best fit the morphological appearances in this case.”

The diagnostic summaries were coded by whether participants referred to the oxidative haemolysis (usually suggesting G6PD deficiency as the cause) and whether they also summarised the abnormal lymphoid disorder. As this is the only case where participants gave their assessment of their own skill level at morphology reporting the

diagnostic summary is shown against the skill level they indicated in the MCQ (see section 4.5.3).

4.5.3 Skill level selected

Multiple choice question: Following feedback from last year's participants' symposium and to help us with future film selection and question setting we would like to know which general statement best describes your day to day activity.

Anticipated answer: It was assumed that the majority would be BMS, but it was not known how many, if any, clinicians participated in the scheme. The question also enabled us to see the proportion of non-reporting biomedical scientists who participated in the scheme for CPD purposes only. An option to allow participants to retain total privacy was also included.

Table 4.19 MCQ: Participants self-selected skill level

Participants selection	Number	%
A/ Biomedical scientist issuing unsupervised reports (R-BMS)	505	65.0
B/ Biomedical scientist issuing supervised reports (S-BMS)	119	15.4
C/ Biomedical scientist. Non-reporter. (NR-BMS)*	58	7.5
D/ Medic issuing unsupervised morphology reports	14	1.8
E/ Medic issuing supervised morphology reports	15	1.9
F/ Medic non-reporter	2	
G/ None of the above	16	2.0
H/ Prefer not to say	43	5.6

*NR-BMS included participants stating they were in managerial roles, BMS working in multidisciplinary or non-microscopy areas *e.g.* blood transfusion and BMS not currently practising *e.g.* on maternity leave or left the profession.

As the numbers for non-BMS participants (groups D, E, F, G and H) were relatively low these are not included in the table of comparison with diagnostic summary shown below in Table 4.20

Table 4.20 Diagnostic summary by self-selected skill level

Morphology diagnostic summary	Total	%
Correct. Both lymphoid malignancy and acute oxidative haemolysis	139	17.0
• R-BMS – unsupervised	102	20.3
• S-BMS – supervised for reporting	14	11.7
• NR-BMS – in a non-reporting role	6	10.3
No conclusion or participant stated they were not able to interpret	95	12.3
• R-BMS – unsupervised	46	9.2
• S-BMS – supervised for reporting	22	18.5
• NR-BMS – in a non-reporting role	11	19.0
Incorrect for both white cell and red cell summary or an incorrect summary for one cell lineage with no mention of second condition	96	12.5
• R-BMS – unsupervised	66	13.1
• S-BMS – supervised for reporting	18	15.1
• NR-BMS – in a non-reporting role	12	20.1
Correct summary for oxidative haemolysis (irrespective of white cell)	528	68.5
• R-BMS – unsupervised	369	73.5
• S-BMS – supervised for reporting	71	60.0
• NR-BMS – in a non-reporting role	34	58.6
Correct summary for lymphoid malignancy (irrespective of red cell)	176	22.8
• R-BMS – unsupervised	126	25.1
• S-BMS – supervised for reporting	22	18.5
• NR-BMS – in a non-reporting role	7	12.0
Summary for red cell features only (irrespective of experience level)	184	23.9
• Correct summary of oxidative haemolysis	173	22.4
• Incorrect red cell summary <i>e.g.</i> thalassaemia, hereditary spherocytosis	11	1.4
Summary for white cell features only (irrespective of skill level)	81	10.5
• Correct summary for lymphoid malignancy	26	3.3
• Incorrect white cell summary (<i>e.g.</i> infection, reactive).	55	7.1

Total absolute number and percentage are shown for the 772 participants who completed the case.

Absolute number and percentage for sub categories are given for:

R-BMS –unsupervised = 505 (65% of all participants who completed).

S-BMS – supervised for reporting = 119 (15.4 % of all participants who completed).

NR-BMS – in a non-reporting role = 58 (7.5% of all participants who completed).

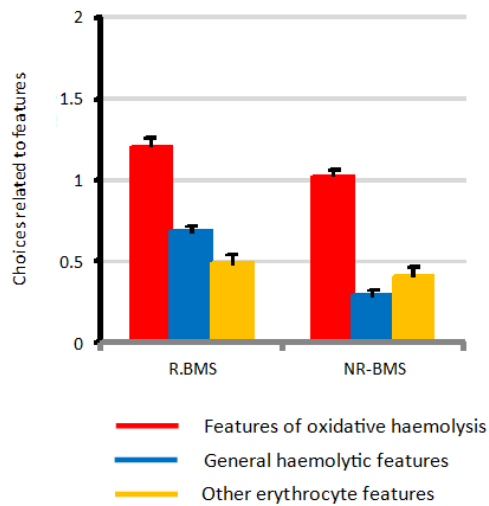
4.5.4 Case 5: summary findings from data

The UK NEQAS(H) DM Scheme for CPD is aimed at BMSs, however, other professionals may register, for this case 47 individuals (mainly clinical staff) stated they had completed the case. The morphology SAG had been aware of the participation of medical staff anecdotally via clinical colleagues but had no knowledge of prevalence before this data. Currently there is still no direct equivalent scheme for medical staff who report morphology.

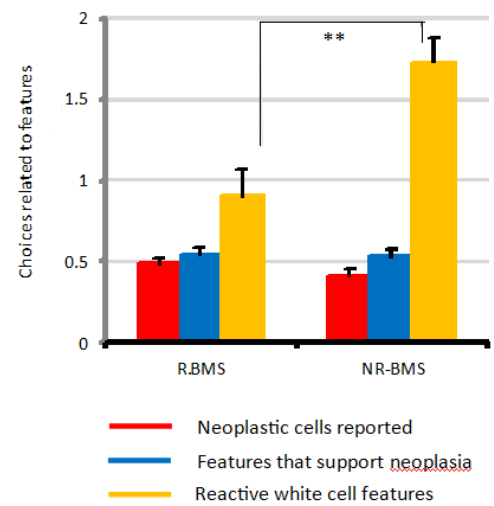
As the non-biomedical staff groups were small, it would not be possible to draw robust conclusions from this data and so they are not considered in further figures. Data in Table 4.20 suggests:

- Those BMS reporting unsupervised appear more able to issue a correct summary report.
- Those BMS in non-reporting roles appear less able to produce an overall correct report.
- Those BMS in supervised roles are less likely to offer a summary report than those routinely reporting.
- Whilst 68.5% correctly identified the red cell abnormality only 22.8% correctly reported the white cell condition.

For statistical purposes two major groups were selected for analysis: unsupervised BMS who stated they regularly issued morphological reports (R-BMS) and BMS who did not work in a reporting role (NR-BMS), the non-reporting group included BMS who specialised in multi-disciplinary teams, other areas of haematology and those with lengthy experience but who had moved in to managerial or other linked careers. Detail is shown in Figure 4.9 panels A to E below:

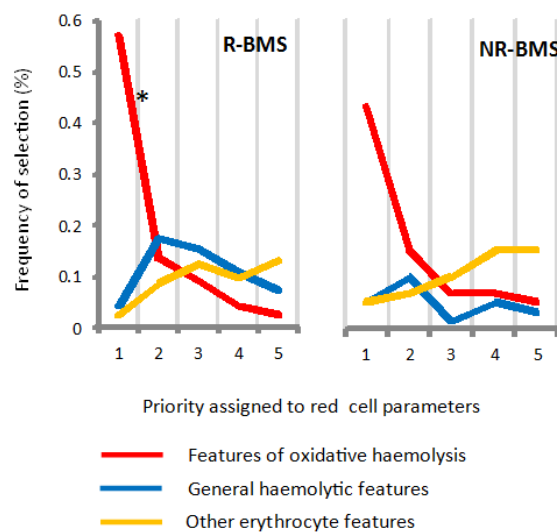


Panel A:
Erythroid features according to skill level

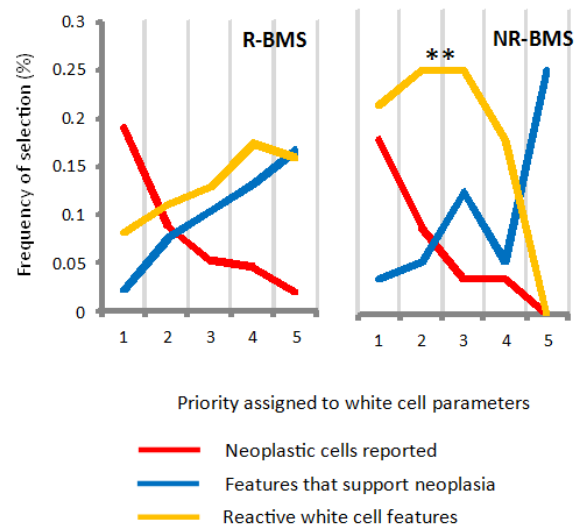


Panel B:
WBC features according to skill level

Comparison of erythroid (panel A) or white cell (panel B) features selected according to the self-selection level of reporting experience, error bars represent SEM. No significant differences were detected in selections for either cell type (Chi-square test).



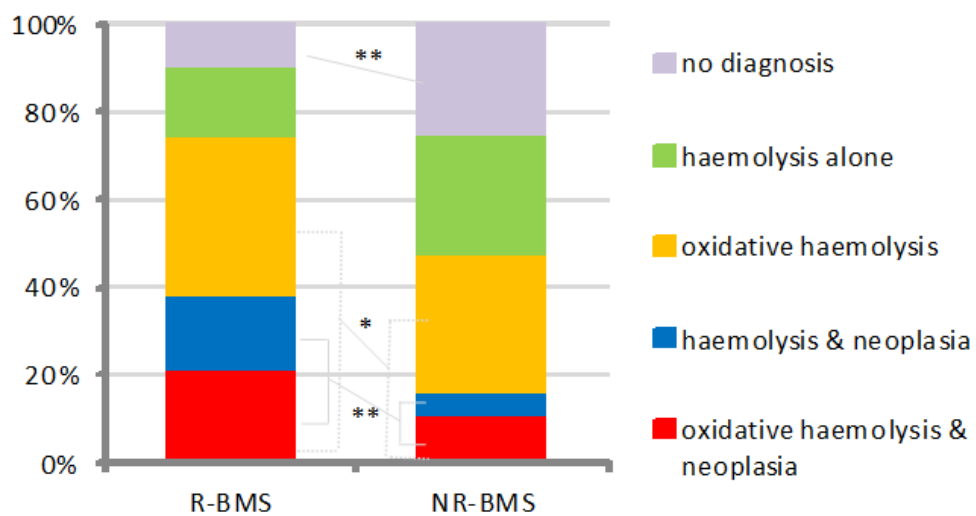
Panel C:
Erythroid priority according to skill level



Panel D:
WBC priority according to skill level

A comparison of the priority assigned to erythroid features (C) or white cell features (D), separated according to the reporting experience of participants. Significant differences are indicated on the plot (Mann Whitney test).

Figure 4.9 Case 5: Analysis of reported features according to the self-selected skill level of participants. Panels A to D. Significance is indicated on Figures 4.9 as follows: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.



Panel E: Comparison between the suggested diagnosis and experience of the reporter.

Figure 4.9 Case 5: Analysis of reported features according to the self-selected skill level of participants, where the morphological features represented two separate pathological disorders (adult T-cell leukaemia/lymphoma and acute oxidative haemolysis). Panel E. Significance is indicated on Figures 4.9 as follows: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ *.**

Table 4.21: Summary data overview from each case examined

Case	Total participant completed (all sections)	Morphological Diagnosis or condition	Principle morphological diagnostic features present on image	Major diagnostic subgroups analysed. Closest to ideal answer shown as group a (Cases 1 to 4).	Group numbers
1	732 (714)	Reactive lymphocytes (EBV virus infection infectious mononucleosis)	Reactive lymphocytes	a. Reactive lymphocytes b. Neoplastic lymphocytes c. Reactive or neoplastic	459 137 52
2	1108 (1028)	Typical Pelger Huët neutrophils (Inherited Pelger Huët anomaly)	Pelger-Huët neutrophils	a. Pelger Huët anomaly b. Myelodysplastic syndrome c. Reactive Changes	584 142 54
3	752 (747)	Microangiopathic haemolytic anaemia (MAHA) associated with viral infection (HIV)	Thrombocytopenia Red cell fragmentation General haemolytic features Reactive lymphocytes	a. MAHA and viral illness b. MAHA c. Haemolysis (other)	125 385 157
4	1011 (948)	Acute myeloid leukaemia (AML) with haemoglobin SC disease (HbSC)	Leukaemic blast cells Target erythrocytes Abnormal erythrocytes (various)	a. Acute leukaemia diagnosed b. Leukaemia not diagnosed c. No white cell diagnosis made	205 459 283
5	789 (772) BMS=682	Adult T-cell leukaemia/ lymphoma (ATLL) with oxidative haemolysis (G6PD deficiency)	Typical abnormal ATLL lymphocytes Changes of oxidative haemolysis affecting erythrocytes	a. Biomedical Scientists regularly reporting blood films b. Biomedical scientists reporting in a supervised role c. Biomedical Scientists not reporting blood films	505 119 58

Note: For Case 5 participants were asked to select a professional category that most represented their daily work from a selection of five alternatives, the three most commonly selected options are shown a. R-BMS, b. S-BMS and c. NR-BMS

Chapter Five: Discussion

The examination and reporting of a blood film is a complex and subjective process performed by an individual who must make a number of assessments in a pressured time frame. They must prioritise these, make a judgement about what the outcome report should state and what, if any, actions are required. Most of this process occurs without the individual consciously or deliberately considering the actual process itself or the skills involved to complete it. Assuming some basic haematology knowledge they:

1. Assess that all cell types are present in appropriate number and in appropriate relationship; this assessment requires the skills of recognition and classification.
2. When abnormalities are recognised the individual must assess the significance of the abnormalities and prioritise them relative to each other. Alongside knowledge of clinical conditions this process involves the skill of being able to appropriately weight findings.
3. In order to produce a succinct and effective report for the clinician the individual will need to distil their findings by interpretation using the skill of decision making. The outcome will depend on the experience and knowledge of the individual. The level of previous experience creates a background expectation which they may use to speed the process but could lead to a wrong conclusion.
4. Additionally there will be a time pressure element of workload waiting and the expectation of colleagues, as the workload between individuals is compared either subconsciously or actually in the form of laboratory and user targets.

5.1 Cases with a predominant single morphological feature

The morphological features present in the image for Cases 1 and 2 (Chapter four, Figures 4.1 and 4.2) affected a single cell type, and were not accompanied by other abnormalities. Irrespective of the diagnosis made, almost all participants correctly identified and then prioritised the affected lineage (97.4% and 83.8% respectively); concentrating their comments on the abnormal lymphocytes for Case 1 and the neutrophils for Case 2 (Figure 4.3 panels C and D). However, within that lineage the

precise classification of the abnormal cells differed significantly between participants in each case. The classification could be divided into distinct subgroups that were linked to the diagnostic summary of the abnormal cell type (Tables 4.2 and 4.6).

For Case 1, those answering the case correctly identified and classified the abnormal cells as reactive lymphocytes, but other subgroups of participants incorrectly classified the abnormal cells as neoplastic, or reported the presence of both neoplastic and reactive cells (Figure 4.3 panel C). Case 2 showed similar findings, with abnormal neutrophils being identified as the most significant feature by almost all participants. Those participants correctly interpreting the case classified the cells as having Pelger-Huët morphology, while those participants incorrectly interpreting the case selected either *pseudo*-Pelger morphology (classing the cells as having dysplastic features and subsequently diagnosing myelodysplasia), or classed the neutrophils as “left-shifted” (assigning a reactive condition as the diagnostic summary) neither of which were correct (Figure 4.3 panel D). Additionally morphological features affecting other lineages were consistently reported depending upon the initial classification of the main abnormal cell. The nature of these additional features was linked to the diagnosis subsequently proposed in each case. In Case 2 (Pelger-Huët) those participants incorrectly diagnosing a reactive process more frequently (but incorrectly) reported reactive changes affecting other cell lineages.

In both cases where a neoplastic disorder was incorrectly diagnosed, participants selected a higher number of other morphological features, but did not identify supporting evidence from other cell lineages. Whereas those correctly diagnosing either case made the fewest additional selections; appropriate the abnormality sat against a relatively normal morphological background.

For both these cases, when examining the outcome from the MCQ actions chosen, participants were influenced by their morphological interpretation, and consequently a diagnosis of neoplasia was associated with a higher need for urgent action (Figure 4.3 panels E and F). Urgent action due to an incorrect conclusion could cause undue concern for either the clinical team or the patient; whilst not as dangerous as missing the important feature, there could still be significant consequences.

These first two cases analysed had shown the importance of recognising and correctly classifying a feature, once classified the feature dictated the outcome action. Interpretation of the morphological features seen is not formally recognised as a function of blood film reporting by BMS, it is assumed they will refer abnormal findings for clinical comment, their interpretation in these cases, however, strongly influenced their subsequent action.

5.2 Cases combining complex morphological features

The next cases to be examined; Cases 3 and 4, had greater morphological complexity so were a greater test of the ability to classify features but also of the importance of prioritising those features to reach an outcome. Case 3 demonstrated a microangiopathic haemolysis accompanied by reactive lymphocytes, reflecting an actual pathological diagnosis of thrombotic thrombocytopenic purpura arising during acute HIV infection (Figure 4.4). Consistent with this increased complexity, participants reported a greater number of morphological feature selections (mean 4.8 from a possible 5 compared to mean 3.9 for Case 2) Table 4.9

Three separate diagnostic groups were identified: microangiopathic haemolysis with concurrent viral infection (the ideal report), MAHA alone without comment of viral features, and haemolysis without specifying a microangiopathic process (Table 4.11). This latter group included various suggested causes for the haemolysis including haemoglobinopathy or nutritional deficiency. The morphological triad associated with MAHA (thrombocytopenia, red cell fragmentation, and general features of haemolysis) was consistently identified by all participant groups (Figure 4.5 panel A) but the major difference lay in the priority ascribed to those features. All groups prioritised thrombocytopenia as the most important aspect of the case (even for those failing to diagnose MAHA) (Figure 4.5 panel B); however, where non-specific haemolysis was diagnosed a significantly lower priority was attached to erythrocyte fragmentation and higher priority to general haemolytic features such as polychromasia. (Figure 4.5 panels C and D).

Participants were less likely to report the abnormal white cells in this complex case with lymphocyte abnormalities reported by only 59.7% (Table 4.10), compared with

97.0% when present as a sole abnormality (Table 4.2) ($p < 0.001$ verses. Case 1, Chi Square test). Similarly, lymphocyte related features were included in the suggested diagnosis of the complex case by only 16.7% of participants (Table 4.11), compared with 97.4% when they were a sole abnormality in Case 1 ($p < 0.001$ vs. Case 1, Chi square test).

In Case 4, an acute myeloid leukaemia arose independently in an individual with an inherited haemoglobinopathy (HbSC disease) causing a plethora of abnormalities affecting red cell, platelet and white cell lineages (Figure 4.6). Unsurprisingly there was a high use of permitted feature selections (mean 4.9 of 5) (Table 4.13) but only a quarter considered both red cell and white cell abnormalities in their summary and only 8.9% were entirely correct (Table 4.15). As the emergence of an acute leukaemia is a critical event, the diagnostic summaries were collated into sub groups. The main three were examined: firstly those who suggested an acute leukaemia, secondly those who noted significant white-cell abnormal features but did not report acute leukaemia, and finally those who did not consider white cells in their diagnostic summary (Table 4.21, Figure 4.7).

As seen with Case 3, the reported red cell features did not significantly differ between the sub groups (Figure 4.7), however, for white cell features the ability to recognise and the subsequent classification of the abnormal white cells was crucial. Participants diagnosing acute leukaemia had reported blast cells with a high frequency, while the presence of blast cells was not (in general) reported by those diagnosing a different white cell disorder or offering no white cell diagnosis (Figure 4.7 panel A). In terms of priority, where blast cells were reported they were correctly assigned the highest level of importance (Figure 4.7 panels B and C), and this was irrespective of whether leukaemia was diagnosed (Figure 4.7 panel D). It was noted whilst coding data that some participants reported blast cells but then did not incorporate them in to a final report. Of the 55.8% who selected blast or neoplastic cells (Table 4.14) 46.1% included them in their summary (Table 4.15). Not drawing attention to such an important feature was surprising but BMS generally report the features they see but might not offer an overall summary, leaving the clinician to draw a conclusion. There was no link

between the abnormal white cell features reported by participants and their interpretation of red cell abnormalities (Figure 4.7 panel E).

5.3 Effect of experience on morphological reporting skills

For Case 5 participants were asked to specify their level of reporting responsibility as a surrogate for experience or seniority. There are no national proficiency standards for reporting of morphology and participants opinion of their own skill levels is open to interpretation; some may have looked at many films but from a mainly well patient population where as others may consider their length of experience low but might have been exposed to a far higher proportion of abnormal blood films.

A complex morphological case was selected for use containing abnormal lymphoid cells of adult T-cell leukaemia/lymphoma together with features of treatment-induced oxidative haemolysis (due to an underlying deficiency of G6PD) (Figure 4.8). It is noted that a case of oxidative haemolysis due to G6PD deficiency had been presented as a DM case previously and it might, therefore, have been expected that some participants would more readily recognise the rare red cell morphology. Indeed this occurred with more than 68% correctly suggesting an oxidative haemolysis in their summary compared to less than 60% previously (DM scheme 1103DM which had no additional abnormal white cell features), although it is not known how many of the same participants had completed both these cases.

Overall the participants made a high number of morphological selections (4.5 of 5), reflecting the large number of features present. A correct diagnosis of lymphoid malignancy was made by only 176/772 (23%), and specifically stating oxidative haemolysis by 528/772 (68.3%), with an entirely correct diagnosis including both disorders made by 139/772 (17%).

For both red cells and white cells, those stating they regularly report films (R-BMS) showed a trend to report features more relevant to diagnosis than the non-reporting BMS (NR-BMS) groups where the features reported were less specific, with a statistically significant difference demonstrated for white cell forms (Figure 4.9 panels A and B $p = < 0.01$).

For prioritisation, R-BMS again demonstrated more effective prioritisation of abnormal forms; this was significant for both red cell and white cell features (Figure 4.9 panels C and D $p = < 0.05$ and < 0.01). The skills of classification and ability to “rank” in order were linked to a higher overall rate of diagnosis both of oxidative haemolysis and of neoplasia by the R-BMS group (Figure 4.9 panel E). However it is noted that, consistent with Cases 3 and 4, some participants from each group failed to identify the neoplastic white cells. Analysis suggested that (at least for the R-BMS group) the features were actually missed rather than the being misinterpreted as reactive cells as there was no increase in the selection of the reactive lymphocyte code when neoplastic features were not selected. This indicates that the reporting skills demonstrated by experienced morphologists were more targeted; although errors were still evident and the pattern of error similar for both groups.

5.4 Understanding the process of providing a morphology report

Improvements to the way morphology education is given should be enhanced by understanding the nature of the reporting process. As each case in this thesis was examined the data raised questions about an individual’s previous experiences producing bias which supported efficient reporting but affected outcomes and which were unexpected. The following table suggests a simple model for the reporting process which could support training in this area.

Table 5.1 A seven-stage model to understand how professionals arrive at a final morphology report

Process	Elements required to complete stage within process
1. Familiarity	Initial assessment of film content, where to start, what to look at.
2. Recognition	Able to assess what appears normal and abnormal
3. Classification	Able to classify abnormalities correctly
4. Reinforcement	Seek for assurance that other features support the classification
5. Priority assignment	Able to prioritise clinically significant abnormalities
6. Interpretation	Understands the significance of how abnormalities are related and can formulate a succinct report which draws the correct conclusion
7. Action	Report outcome leading to the correct clinical action being taken with appropriate communication.

5.5 Reducing the effort when associating observations in context, consideration of heuristic techniques

Judgment and decision making, in a time pressured environment, are integrated into every aspect of laboratory practice. Only when an incorrect action causes an incident do professionals fully investigate how conclusions were reached, in order to prevent reoccurrence of the error.

The techniques our brains employ to arrive at a decision with the minimum of effort have been defined as “heuristic” methods (Simon, 1990; Shah & Oppenheimer, 2008). These are usually a mixture of conscious and sub conscious processes which combine to produce a quick and satisfactory outcome on most occasions. Research has shown that young children are quickly able to differentiate different animals when given pictures; by contrast computers are unable to reproduce the same level of accuracy (Zhang, Sun, & Tang, 2011). Equally people with poor vision can correctly identify people they know in a crowd when all faces and movements are indistinct. This is

because our brains employ “fast and frugal” heuristics enabling us to outperform computers in these complex situations. These techniques, however, may introduce a source of bias, which usually goes unrecognised by the individual, but can lead to mistaken conclusions. In daily life we may glance at someone and think we recognise them but when a stranger turns we have made a mistake in identity recognition. Some mistakes made quickly before all available evidence has been considered can lead to serious error.

Recognition of objects and people require skills based upon two separate but linked processes; the first being the ability to perceive something as familiar or unfamiliar (this is a rapid intuitive process based on previous experience). The second process is the skill of recollection (a slower conscious recall of learnt knowledge) (Henson, Rugg, Shallice, Josephs & Dolan, 1999; Wagner, Desmond, Glover & Gabrieli, 1998). In everyday life there are countless situations that require prompt action without much deliberation, in which experience of a situation is integral to making a quick decision.

5.6 Heuristic techniques in morphology reporting

Morphology reports have a key diagnostic role; poor quality reports, over reporting of non-critical features or missing of important features can be queried by clinical teams, picked up by senior staff checking reports or detected by other staff reviewing test results. Those involved learn from these experiences which will influence their future decisions when reporting whether they realise this or not. Such trial and error techniques of learning may be considered heuristic. In order to complete each morphology report the individual will consciously and subconsciously apply various techniques or strategies which simplify these complex processes. They are unlikely to realise that they are using heuristic methods to interpret the morphology and complete their report, but it is the very techniques the morphologist employs to make fast and safe decisions that may also lead them to produce poor or misguided conclusions. The level of impact for the patient may be low, although morphology reports are an important diagnostic tool, so an incorrect or misleading report may be distressing for the patient but could also be catastrophic should an acute clinical condition be missed.

The question of how a heuristic approach impacts on blood film reporting has never been assessed, however, the examination of participant submissions enabled analysis of the responses of many hundreds of professionals who report blood cell morphology as a routine part of their practice. In doing so it was possible to analyse their ability to arrive at correct or incorrect conclusion for a range of representative cases and to detect common patterns of bias. Acknowledging the varied levels of experience the examination has revealed common patterns of approach to interpretation, but has also highlighted patterns of error shared by the different groups of participants. These data have relevance to indicate how support mechanisms could be better designed to improve the interpretation skills of individuals who report haematological morphology.

As the examination of data from each case was undertaken it was intriguing to see how the morphological features selected were then interpreted to give a succinct final report and it became noticeable how the outcome report strongly influenced the actions taken. Due to the very nature of microscopy, morphology reporting is a largely unregulated process; those performing it are working in a pressured environment and must make their decisions efficiently but safely. They must be able to recognise that an abnormal feature is present and then be able to recall from their experience the correct classification. When reporting blood cell morphology familiarity is likely to drive the initial recognition of any abnormality, and in this research there was generally substantial agreement on the affected cell lineage irrespective of any conclusion reached. However, subsequent evaluation of the chosen classification made by participants of the abnormality they perceived requires active recollection, and this conscious process revealed an interesting variability.

For Cases 1 and 2 where a single abnormal feature was present the majority recognised the abnormality but far fewer correctly classified this abnormality leading to correspondingly different outcomes chosen for further action. The data also showed that abnormal erythrocyte forms were more often classified correctly and, therefore, reported in consensus agreement but the classification of abnormal white cells varied markedly between participants. It is probable that the ability to classify red and white cells is influenced by the very nature and appearance of these cell types. Abnormal erythrocytes are often present in large numbers so our brains see multiple similar

occurrences re-enforcing our decision whilst they are simple forms, usually geometric and without much central structure, it is a fact that such shapes are known to be rapidly and accurately recognised and classified by the human brain (Larson, Aronoff, Sarinopoulos, & Zhu, 2009; Bar, 2003). By contrast, abnormal white cells are usually relatively infrequent so, it is less likely that examples will be viewed directly together, they are also individually complex containing both nuclear and cytoplasmic structures. When the Cases 1 and 2 were originally prepared for the DM scheme care had been taken to ensure the images contained sufficient examples of the relevant classic abnormal white cells required. The assessment of abnormal white cells requires us to recognise subtle changes of specific features, our ability to achieve this will depend strongly on conscious evaluation (Di Carlo, Zoccolan & Rust, 2012). However, when considering all cases the more consistent classification of erythroid features seen by participants failed to translate into an improved morphological conclusion, suggesting that additional factors were also involved in distilling their findings into a diagnostic summary.

Irrespective of the nature or frequency of any abnormalities present in a blood film, the data obtained through morphological assessment by microscopy is inevitably complex. Each of the five DM images examined by the scheme participants comprised 50 to 90 separate fields and so depicted 3000-4000 cells giving many possible morphological descriptions to consider. In Case 5 (Table 4.17) participants made 63 different morphological selections (of 74 available). Distilling a conclusion from this complexity presents the morphologist with a problem as there are simply too many different cell features for each to be considered individually. This was confirmed by the heat-map exercise in chapter 2 (section 2.7.1) showing that users viewed images without following a set protocol but went immediately to features of interest. Professionals reporting blood films must arrive at a conclusion in a pressured time frame but that conclusion needs to be as accurate and informative as possible, so to achieve this, strategies that simplify and direct the analysis of the film must be employed. Specific conscious evaluations, such as cell classification and prioritisation of abnormalities, are central to the analysis; additionally the often unconscious mechanisms of heuristics are employed. These techniques may be highly effective at supporting fast and frugal decision making (Goldstein & Gigerenzer, 2002), or may

introduce bias (Croskerry, 2013; Dawson & Arkes, 1987). Heuristic processes have been widely reported in other areas of medicine (Klein, 2005, Crowley *et al.*, 2013; Wegwarth, Gaissmaier & Gigerenzer, 2009; Murray *et al.*, 2015) and are recognised to be prevalent in image based interpretation in radiology, (Gunderman, 2009) but have not been studied in laboratory haematology before. Considering the findings from the analysis of the DM cases in this thesis heuristic processes appear to be potentially relevant. They should, therefore, be considered in the study of haematological morphology as summarised along with their associated cognitive biases and are summarised in Tables 5.2 and 5.3 (derived from Shah & Oppenheimer, 2008; Tversky & Kahneman, 1974; Blumenthal-Barby & Kreiger, 2015).

Table 5.2 Summary of the processes used by participants enabling them to complete a morphological report

Panels A to D. Table includes examples of the linked forms of error or bias that arose:

A. <u>Classification</u> (knowledge-based decision making to determine a data set)	
Classification (simplification function)	Assigning related observations to a discreet `class`. The cells comprising that class are then considered together as a single diagnostic feature. Example: the class 'target cells' can be related to other erythrocyte classes such as basophilic stippling, microcytosis and hypochromia to be a combined larger class of related observations 'features of haemoglobinopathy'.
Classification (directing function)	Using the known pathological significance of an identified class to direct examination to seek evidence of a specific disease state. Example: the presence of target cells may direct a search for macrocytosis, thrombocytopenia and stomatocytes suggestive of liver disease or for microcytosis and basophilic stippling suggestive of a haemoglobinopathy.

B. <u>Simplification heuristics</u> (techniques employed to reduce the complexity of datasets)	
i. Weighting	<p>Attributing a relative importance to each individual class based on the perception of its diagnostic significance.</p> <p>Example: frequency of forms belonging to that class (target cells might be of importance if numerous rather than occasional), or the perceived pathological importance of the features (occasional fragmented red cells may be considered of more significance than occasional target cells).</p>
ii. Elimination	<p>Using the class assigned, and the weighting attributed to them to identify those features considered to have low importance to diagnosis and excluding them from further analysis.</p> <p>Example: if occasional target cells are considered of low significance they will simply be excluded from the features selected for reporting.</p>
iii. Sources of bias	<p>a. Bias of imaginability: complex findings are simplified by the morphologist to remove elements considered less important for diagnosis: this may not follow objective criteria.</p> <p>Example: complex red cell morphology may be distilled to two or three elements suggesting differing pathological processes.</p> <p>b. Inattention error: being distracted by multiple elements in a complex picture, therefore failing to notice or consider specific important features.</p> <p>Example: in complex cases the morphologist may fix on a key feature such as the presence of a haemolytic picture and entirely miss the presence of leukaemic blast cells.</p> <p>c. Associative thinking: belief that events occurring together are likely to be linked; leading to placing unlinked observations into a single class.</p> <p>Example: Linking extreme thrombocytopenia as a feature of a haemolytic process so missing that there is bone marrow failure due to a concurrent leukaemic process.</p>

C. Context heuristics (actively seeking other data that supports a particular decision)	
i. Framing	<p>Reinforcing a diagnostic impression through the identification of supporting features or other classes that are consistent with the same pathological process.</p> <p>Example: seeing Pelger-Huët cells and interpreting them as left shifted neutrophils then looking for toxic granulation in order to support the conclusion of a reactive process.</p>
ii. Availability	<p>Interpreting features based on a perceived likelihood for a particular disease process.</p> <p>Example: taking account of the clinic of origin, age of patient, previous experience.</p>
iii. Sources of bias	<p>a. Framing bias and inattention error: the preconceived diagnosis is inappropriately favoured by overemphasising features that support the diagnosis and giving less weight to features that do not fit.</p> <p>Example: considering a haemoglobinopathy (when one is not present but a haemolytic event is) may report target cells even when numbers are not significant and ignore the presence of more frequent hemi-ghost cells.</p> <p>b. Availability bias: “common things are common” so less likely explanations are given less consideration.</p> <p>Example: spherocytes are associated with haemolytic anaemia so the irregularly contracted cells seen in a G6PD deficiency haemolytic crisis may be misinterpreted as spherocytes leading to an incorrect report.</p> <p>c. Anchoring bias: becoming rooted in an idea (e.g. “this is a leukaemia”) this interpretation is then maintained even where no further evidence is present. Anchoring is associated with “loss aversion”: observations with high potential significance, such as “features of possible neoplasm” are not eliminated even where the observer considers the diagnosis unlikely. Example: participants selecting blast cells as a top 5 feature but then not considering them in their summary reports.</p>

D. <u>Completion heuristics</u> (techniques that support completion of task) A report must be issued and the mind cleared for the next report.	
i. Attribute substitution	<p>Simplifying the task. Changing the question from the objective “what is the diagnosis?” to the subjective “do the features fit with my preferred diagnosis?” Loss of “open mind” rather than reporting what is seen or if unable to interpret findings but needing to make a conclusion fit.</p> <p>Example: some individuals found features to support a conclusion of myelodysplastic syndrome in the Pelger-Huët when none were present.</p>
ii. Sources of bias	<p>Premature completion of task. When a conclusion is reached that is deemed sufficient to stop examination (‘satisfying’), this may lead to a premature conclusion before all evidence has been discovered.</p> <p>Example: having successfully reported the extensive red cell features of the haemolytic process from a G6PD deficiency crisis some individuals concluded their report without considering any white cell features and so completely missing concurrent malignancy.</p>

Cases 1 and 2 reflected disorders that affected a single lineage with no accompanying abnormalities and the error pattern differed from those in more complex cases. Essentially, knowledge-based skills of recognition and classification had primary importance and the subsequent diagnosis and actions taken were strongly linked to the choice of cell classification. The data also suggested that participants additionally sought to support their decision through contextual heuristic processes (Table 4.21 and Table 5.2). In particular, incorrect diagnoses were associated with reporting a greater number of morphological feature selections and with evidence of biases associated with seeking supportive context. Experience suggests that the novice morphologist tends to report more features, fearing to miss an important feature, anecdotally over-reporting, in part because their skills of interpretation are limited.

For participants who favoured a *reactive* diagnoses the use of “framing bias” led to the incorrect reporting of reactive features in other lineages that were not present in sufficient number to warrant reporting or were simply not there. Those participants concluding a *malignant* diagnosis were “anchoring” to a neoplastic interpretation despite lack of supporting evidence, and with “loss aversion” where participants reported both reactive and neoplastic features on the film but then did not question the clinical likelihood of their findings (summarised in Table 4.21).

Where the DM images were of complex combinations (Cases 3 to 5), the participants analysis predominantly employed strategies that simplified the observations. The knowledge-based stratification of findings and the elimination of less important features allowed many participants to deliver more relevant interpretation (hence even though many different features were selected, participants were often able to correctly narrow their options to the most clinically relevant). Despite this, errors related to prioritisation were found. For example in Case 3, there was widespread agreement, with the presence of thrombocytopenia as the most frequently selected feature, although some participants entirely failed to consider the low platelet count in their interpretation. Importantly some participants gave a low priority ranking to the key feature of red cell fragmentation, resulting in an incorrect conclusion of general haemolysis rather than the more specific diagnosis of microangiopathic haemolysis. If that were a real patient situation the importance of providing the right information in

an urgent manner to the clinical team is imperative; as the haemoglobin concentration may be normal. Poor prioritisation of multiple features will dilute the specific nature of the haemolytic process and potentially detract from a conclusion of acute haemolysis. The question of whether participants had sufficient background training in haematology to correctly prioritise thrombocytopenia and red cell fragments was unknown but those actively reporting morphology have usually already gained general haematology experience authorising FBC results. The presence of the keratocytes and fragmented red cells are specific to the mechanical nature of the acute haemolytic event, reporting these features adds valuable clinical information that the medical team cannot determine from the numerical values of the FBC parameters alone.

Significant error also arose from the application of simplification heuristics (Table 5.2 panel B): “Associative thinking” led to very significant morphological features being assumed to form part of an existing class rather than having independent significance. This could have minor effect *e.g.* the failure to include viral disorder in the conclusion to Case 3 where the clinical urgency would be communication of the haemolytic process. Failure to recognise two unrelated condition could also have a major effect, notably when participants failed to include leukaemia in the conclusion to Case 4 (haemoglobinopathy) even when some had identified that blast cells were present and as a consequence would not have referred the film for urgent clinical review.

Biases associated with the need or desire to complete their examination were also seen (Table 5.2 panel C): In Cases 4 and 5, a significant group of respondents failed to report the presence of neoplastic white cells. These errors were likely to derive from the complexity of the features and the requirement to finish the case, resulting in “inattention error” or the associated bias “premature completion of task” (summarised in Table 4.21). Having found a series of complex features it is possible some participants thought they had “found the abnormality” and discarded the mantra of blood film reporting “red cells, white cells and platelets, never forget to examine all areas”. Ignoring this mantra can also lead to the same error in a real patient situation.

Table 5.3 Morphology reporting skills and the types of error or bias shown in the five cases studied

A. Cases 1 and 2 (simple morphological features) Case 1: Viral infection related reactive morphology Case 2: Pelger-Huët anomaly with all other morphology normal.		
Primary skills: Recognition and classification Relevant heuristic group: Context		
Error group	Incorrect classification. Source: Knowledge/skills-based error	
Supporting bias 1	Case 1: Overemphasis of reactive features to support a reactive diagnosis. Source: Framing bias	
Supporting bias 2	Cases 1 & 2: Favouring malignant diagnosis without supporting evidence Source: Anchoring bias and consequence bias	
B. Cases 3 to 5 (complex morphological features) Case 3: MAHA in a new case of HIV positive viral infection. Case 4: Acute myeloid leukaemia arising in a Haemoglobinopathy (HbSC) Case 5: Oxidative haemolysis of G6PD deficiency in a case of ATLL being treated.		
Primary skills: Recognition classification, prioritisation Relevant heuristic groups: Simplification and completion		
Error group	Incorrect classification. Source: Knowledge/skills based error	Cases 4 and 5
Error group	Incorrect prioritisation and interpretation. Source: Knowledge-based error	Case 3
Supporting bias 1	Failure to report significant feature. Source: inattention error, premature completion of task	All Cases
Supporting bias 2	Failure to include an observed feature in the interpretation: Source: associative thinking, attribute substitution	All Cases

The reporting responsibilities and experience of participants in the UK NEQAS(H) DM scheme for CPD varied. However, blood film analysis is always interpretive even when the decision concerns whether (or when) to seek a colleague's help. Cases 1 and 2 clearly showed that the action taken in response to morphological appearances is tightly linked to the perceived diagnosis. In both those cases participants had a simple but key question to consider; is the abnormality malignant or not with the outcome action being totally different. Accuracy is an important facet of post-analytical quality ISO 15189. This research showed that while cell recognition is an important part of this process, the techniques of blood film interpretation also depend on effectively using a range of heuristic methods. These methods are hugely valuable in driving effective conclusion, and the data from Case 5 suggests that experienced morphologists apply these unconscious techniques more accurately than those with less experience. However, biases related to heuristics are also evident with the experienced group whose reports are less likely to face a second review. With internet-based material so widely available individuals can seek their own support (Ceelie *et al.*, 2007; Lee *et al.*, 2013; Crowley *et al.*, 2013) it becomes essential that we actively seek to understand, and address how we arrive at rapid accurate conclusions and where the errors occur (Hamilton, van Diest, Williams & Gallagher, 2009).

The complexity of some of the cases studied were beyond that which most morphologists would see in day to day practice, although all cases were genuine and so could have presented to most laboratories. The image size was limited and the viewing experience not equivalent to that of examining a blood film so the findings in this thesis have to be considered with caution. It does appear, however, that heuristic processes do assist the knowledge base of the morphologist when producing a report so should be considered when designing training and morphology support. Semi-automated cell recognition systems are being increasingly used in laboratories, these can produce auditable reporting and certainly reduce errors around cell classification but without acknowledging the complexity of the decision making process those reporting are still vulnerable to bias and error.

5.7 Digital images and morphology education beyond the UK NEQAS(H) scheme

There are many respected haematology professionals now translating their teachings from conventional to digital formats. Notably the interactive ImageBank available on disk by Professor Barbara Bain (Bain, 1999) and Gillian Rosenberg, a respected scientist practising in Australia, has released an on-line teaching package which is related to her previous conventional publications and is validated by the Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP, 2016). Unlike the UK NEQAS(H) DM scheme for CPD, these products contain mainly single frame images rather than large stitched images.

There are training packages designed for use via the internet such the French led e-hematimage training program which has translations in eight languages (e-MEDICINimage, July 2017). With professionally validated educational information this system uses single images in composite form (not stitched) thus its main attraction is for those wanting to learn white cell differential skills. CellaVisionTM, the largest supplier of DM technology for laboratories across Europe, publish an on-line blog of interesting morphological features for others to comment upon (CellaVision, 2017a). In addition, CellaVision have published an atlas application of unstitched images (CellaVision, 2017b) which provides images of mainly single cells and so concentrates on white cell morphology and this technology forms the basis for a digital morphology EQA service in Sweden. As nations start to include the use of digital images in their own blood cell morphology EQA services, as seen in Spain, they must consider both the technical aspects and quality aspects of their services to engage with participants (Gutierrez, Merino, Domingo, Jou & Reverter., 2008).

The internet has increased the opportunities to access training material but there considerations for users when making their choice; these include subscription costs and, for international options, spelling and terminology. The ICSH has recently published guidance on morphology nomenclature, (with supporting single feature images available on-line) although unless national professional bodies raise awareness amongst their membership standardisation might be slow (Palmer *et al.*, 2015).

Digital images are already being used to support professionals across geographic divides, the International Network for Cancer Treatment and Research community (INCTR) use the telemedicine system by iPATH™ for users to upload images of bone marrow smears in order for colleagues to share, view and pass opinion (INCTR, 2017). Whilst keen to state that professionals are not providing a diagnosis their opinions may influence the outcome of the final report. This system, arising from the European bone marrow working group in Basel, is available internationally to support professionals who need expert advice, any professional can register and upload an image of a film and ask for expert comment. The DM team has also worked on projects with the World Health Organization using images to support the education and quality of malaria morphology reporting in Africa (Tatum *et al.*, 2008; Ahmed *et al.*, 2016) where there are considerable challenges for meeting user needs. Understanding what professionals want as a user experience is just as important as providing good quality images.

That users of the UK NEQAS(H) DM scheme for CPD appreciate the quality of the product is confirmed by their continued participation. Although, some aspects of how they actually respond to the cases was unknown, this work has shown that an understanding of the reporting process needs to be considered when developing the service further. User experiences with digital imaging for morphology are now well established, although this is the first time those experiences have been examined in depth for a large user group. The findings from this work should be considered by those striving to educate professionals who report blood cell morphology.

Chapter Six: Conclusion

The modern laboratory faces ever greater pressures for efficiency as service users also face the requirement to diagnose and treat patients quickly. Within the 24/7 working environment the important skill of reporting blood cell morphology must occur in a timely manner and cannot be left for those perceived as experts working other shifts. Reporting of blood films must be carried out at nights and weekends, by those who may cover multi-disciplines and may not even be haematology specialists. As labour intensive methods of teaching morphological skills become less widespread, the computer-driven decision-support mechanisms are becoming more widely available. Laboratories and the staff reporting morphology have no choice but to grasp new technologies to support their learning and so those providing training, or managing laboratories, must understand how best to support staff.

In this thesis the responses of a large number of laboratory professionals, who examined the same blood films using a virtual microscope, were explored. It was found that the participants supported their knowledge-based decision making process by incorporating a range of additional techniques that helped reinforce or simplified their analysis. These “heuristic” approaches were used subconsciously in a similar process to those used in many other areas of human decision-making in order to support rapid accurate decision making about everyday situations. However the very techniques used to help make fast and precise decisions may also lead to an unrecognised bias and are a source of error. Moving forward with the UK NEQAS(H) DM scheme for CPD the morphology SAG will need to consider these effects and incorporate decision-support systems to identify and reinforce the positive aspects of these skills, while seeking to minimise associated biases.

Participants of the UK NEQAS(H) DM scheme undertake cases to show evidence of CPD and do so under uncontrolled conditions using an internet-based image system different to reporting blood films by microscopy. Undoubtedly there will also be differences in their attitude to completing CPD compared to their approach to patient blood films. For this research, however, the numbers participating are large and with no equivalent studies on blood film reporting using microscopy the outcome data is

important. This work has shown that skills of white cell and red cell identification differ (with erythrocyte identification being simpler and therefore easier to learn) and that simple and complex films are examined differently with different heuristic tools applied. Reporting accuracy in Cases 1 and 2 was relatively high, which offers reassurance to participants, those managing the laboratory service and to patients; that said reporting accuracy should have been high for Case 1, which depicted a condition that every individual reporting blood films will have seen in a patient situation. The latter cases studied depicted rare conditions which would not normally be encountered by the majority of this participant group, so the error rate might be expected to be high. The size of the images used is not directly comparable to viewing a whole blood film and so could, therefore, be criticised as unrepresentative of routine practice. Accepting that these factors may have added to the errors made and that the findings cannot be literally translated to the microscope on the laboratory bench, this study has provided an unequalled insight in to the reporting of morphology by a large number of laboratory-based professionals. Despite some limitations of the data analysis a lower accuracy for identifying morphological features was linked with evidence of heuristic, rather than knowledge-based, error although the correct outcome action (to refer to a senior colleague) was almost exclusively taken in these cases.

The data demonstrate that mechanisms of error associated with heuristic processes are highly important in morphological decision making. Decision-support mechanisms and training interventions must recognise the different facets of achieving a morphological diagnosis, and actively address both the knowledge-based and heuristic processes that combine to drive rapid and accurate decision-making. There is a significant part to play for effective decision support tools in blood film reporting but these must be easily accessible and understandable to users. With limited time allocated to training, the erosion of one to one teaching and an increased reliance on internet-based information, the new morphologist is less likely to reach for a text book and more likely to access the internet for training support material.

Although there has been a growth in automated cell-recognition systems, aimed primarily at white cell classification, the national uptake of these systems in routine

use, even by the large automated laboratories, is slow. These systems may reduce errors associated with premature completion and inattention (human traits), however, reporting a blood film effectively requires the integration of multiple facets of the morphological features and considers how the information is refined down to a range of diagnostic outcomes. Equally the automated systems classify white cells but a trained human then needs to interpret the significance and the relationship of the features seen and formulate this in to a succinct report.

6.1 Research in context

Without ensuring staff reporting morphology are proficiency tested, or training provided to standardise the format and quality of the morphology report itself, it will be a challenge to move the DM scheme from pure CPD to a scored proficiency scheme. UK NEQAS(H) has yet to introduce a robust scoring element to the conventional glass slide scheme, although as a UKAS requirement, it should be considered that future developments for DM include a proficiency element. Indeed, when participants of the UK NEQAS(H) annual symposium in York 2014 were asked whether they would be prepared to see skill levels introduced to the DM scheme the response, by a show of hands, was overwhelming positive. Caution has to be shown when translating the findings from this research to blood film reporting via microscopy, however, the exercises using images prepared from blood films previously distributed in the glass slide scheme gave comparable results; suggesting that these research findings are relevant to routine laboratory practice.

It is important to remember that the number of semi-automated cell recognition systems in use is slowly increasing and the morphology SAG are considering whether an EQA scheme for these systems is also required. The use of digital images in morphology reporting is gaining momentum and quality assurance for their use is required.

Laboratory-based BMS are not alone in needing new initiatives to demonstrate proficiency in blood film reporting; clinicians working in pathology laboratories must also consider how they demonstrate, to the public, that they are maintaining and raising professional quality standards as the debate over revalidation for pathologists

continues. The UK NEQAS(H) DM scheme was not aimed at clinical haematologists yet 29 chose to participate independently (Case 5) and it must be acknowledged that they have no nationally accepted method for collecting evidence of their own skills for reporting morphology.

Importantly, despite UK NEQAS(H) only promoting the DM scheme to BMS in the UK, the use of the internet allows access to the system internationally and this has been exploited by some non-UK based professionals. There are quality assurance and educational schemes, using digital morphology, in other countries *e.g.* Spain, Sweden, Australia and the United States of America, so the data from this research has relevance to a specialised international audience. This data can help inform education of morphologists, develop proficiency and improve standards of morphological reporting both abroad and in other allied professions.

6.2 Future work

The UK NEQAS(H) DM scheme for CPD must evolve to stay relevant to current participants and to consider new opportunities. The IT system housing the images is being re-developed, taking into account the lessons learnt from this research, with a re-launch planned for April 2018. One objective is to link the morphology cases to improved educational content recognizing the common causes of error, the narrative will be strengthened to advise morphologists on possible bias in their summary reports. By explaining to participants the common patterns of error seen it may be possible to positively influence their future reporting and, in doing so, improve morphology reporting nationally for patient care.

Future developments of the scheme also include the introduction of leucocyte differentials, whilst the introduction of skill level comparison groups has not been ruled out, that could be a first step towards individual performance assessment for blood cell morphology reporting.

Chapter Seven: Reflection

Following 12 years working in a stem cell therapeutics laboratory, with protected time for research, I accepted my current managerial position with the encouragement that a research element to my role would be welcomed. Initially I was able to develop my long held interest in blood cell morphology which, led to an invite to the IBMS Haematology Advisory panel and also to lead the morphology session at the bi-annual congress. Professor Hyde, Director of UK NEQAS(H) saw the potential of emerging internet-based technologies and asked me to chair meetings of a small team of interested professionals in Manchester to investigate possibilities for incorporating digital images in education for participants. The digital morphology project evolved by working with and for UK NEQAS(H) to create exercises participants and then present the findings back to them at the annual symposia.

I underestimated the personal commitment that would be needed from me, to fully commit to this work, whilst employed in a busy role with substantial demands on my time. The ever increasing drive for efficiency in the NHS puts particular pressures on activities beyond those required to provide a routine service and this pressure continues to increase.

Collaborating with UK NEQAS(H):

The reputation of UK NEQAS(H) could not be placed at risk, every step of the development (chapters 1 and 2) involved a small team, was discussed in meetings or teleconference and then had to be agreed at the morphology SAG. Some developments moved more slowly than I would have liked or were greeted with less enthusiasm than anticipated, but each step and every proposal had, quite rightly, to be considered by national experts and those responsible for the good reputation of UK NEQAS(H). I learnt patience and that accepted wisdoms could be challenged and that when the evidence was found, robust support was available and progress was assured.

When preparing the final case reports for the DM scheme it seemed to me there was a lot of data that was not being fully considered, notably the free text responses allowed participants to comment on any and all aspects of morphology reporting. This was an opportunity to examine part of the DM scheme away from the collaborative process

but under the regulation of UK NEQAS(H). At that point I had was unsure as to what the data would show, however if the outcome would improve the scheme for participants I would have considered the process worthwhile.

Getting authorized agreement for the extraction of data from the scheme was, justifiably, a protracted process and a nervous time for me. The UK NEQAS(H) Manager and Director (and the morphology SAG) had to be confident that no information could be traced back to individuals. Should the process have caused any issues, ethical or technical, at any point then the project would have been cancelled. Getting the original agreement took more than a year and data extraction occurred under close scrutiny.

Technology:

I did not have any technical expertise as far as photography was concerned and initially found the process of stitching, colour correcting, manipulating images, creating annotations and attaching narratives, stressful and laborious. Hours could be spent creating an image that might not be approved for use. I found, however, letting someone else annotate an image I'd prepared just as difficult, experts volunteering to build cases invariably underestimate the care and time needed, so the creation of cases has remained primarily at CMFT with John Burthem and myself.

Data handling and analysis:

When the data was extracted, the format was not exactly user friendly, and it took many hours to simply get the data aligned for each case prior to coding. Coding (due to the high number of participants) was laborious but fascinating as clear patterns of reporting emerged. Coding of the free text proved extremely challenging, I was astounded that there could be so many ways to reach a conclusion and shocked and intrigued by some of the outcome reports. The very solitary nature of providing a morphology report by microscopy had not enabled investigative study of this process, so it was important to regularly discuss the findings, as they emerged, with the Consultant lead. This enable me to approach the SAG asking to release case data for the complex cases and to ask UK NEQAS(H) to ask participants to select a skill level for one case.

My stress level increased on finding the statistician at Portsmouth left precisely as I arranged to see him and I failed to find the access I needed from his successor. Being a distance student can be problematic when regular or intense discussion is needed. The statistician at CMFT, who had offered support, then took a post elsewhere and it took Dr Burthem considered time and effort to ensure statistical support locally. Ensuring that the statistics and data examination was robust was essential but again delayed the project.

Making a difference:

I have never received payment for my role creating and producing cases for the DM project although the work has enabled me to attend meetings with like-minded professionals without seeking funding from my employer. I've felt privileged to have represented UK NEQAS(H) DM scheme internationally and had to become familiar with the workings of the organisation to do so. I have carried out most of the work outside of my employed hours in order to have the professional freedom and control of my input to NEQAS(H). This has taken a time commitment that I could not have given without the support of my family. Most professionals cannot or will not put aside such a quantity of time for extra work activities. Involvement in developing my profession remains the most exciting part of role. Challenging the unknown may have less formal responsibility, but it has been more rewarding.

Debating the issues that surround reporting blood cell morphology has placed me in an arena that is predominantly that of the clinical haematologist and so I've had to justify my opinions and the role of the laboratory scientist, this ensured I had considered all aspects and I have generally enjoyed the challenge.

I have never considered myself ambitious but I am passionate about the laboratory haematology work carried out by BMS and providing a quality service for patients. The DM project gave me an opportunity to be involved in developing something that has impacted positively on my profession nationally and internationally.

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Appendix A: UK NEQAS(H) ethical form for sample collection



United Kingdom National External Quality Assessment Scheme for General Haematology

Organiser: Dr J Parker-Williams
Manager: Mrs B J De la Salle

Telephone: (01923) 217878
Fax: (01923) 217879
Email: haem@ukneqas.org.uk

UK NEQAS(H)
PO Box 14
WATFORD
WD18 0FJ

CONSENT FORM FOR DONATION OF BLOOD OR BONE MARROW FOR PERFORMANCE MONITORING AND EDUCATION.

Dear Patient,

Your Consultant has requested that as part of your clinical investigations, you should have a small amount of blood or bone marrow taken so that it can be examined to help with your treatment. Looking at cells from the blood or bone marrow, where blood cells are made, is a very valuable approach to reaching a diagnosis in blood disorders.

UK NEQAS for General Haematology provides a service to haematology laboratories, mostly in hospitals, to enable them to achieve continuing high standards of professional excellence through education. To maintain these standards, we send them samples from a patient such as yourself, which they examine, report on and return their results for us to analyse.

We would be very grateful if you would agree to allow any remaining blood or bone marrow sample to be used for this purpose, once all the tests requested by your doctor have been completed. It does NOT mean that the procedure has to be repeated. Also, I can promise you that your identity will not be revealed; we use coded numbers which are known only to myself.

If you agree to this please could you complete and sign the slip below. This will be returned to us for our records and a copy kept in your hospital notes.

Yours sincerely,

Dr J Parker-Williams
Scheme Director UK NEQAS (H)

CONSENT FORM FOR DONATION OF BLOOD OR BONE MARROW FOR PERFORMANCE MONITORING AND EDUCATION.

I have read the information above and understand that any remaining blood or bone marrow sample, collected for diagnostic reasons, may be used by UK NEQAS (H) for the provision of educational material for examination in haematology laboratories. I understand also that my identity will be known only to the Director of the UK NEQAS(H) Scheme and will not be disclosed to the participating laboratories.

Signature:.....

Please print name here:.....

Signature of Doctor taking blood or bone marrow sample:.....

Please print name here:.....

Date:.....



WHO COLLABORATING CENTRE FOR QUALITY ASSURANCE IN HAEMATOLOGY
CENTRE FOR INTERNATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME



CPA(EQA)
ACCREDITED
SCHEME

FOR INFORMATION USE/Consent form for blood & bone marrow samples for film etc

Appendix B: Letter of Ethical Approval and Governance

Central Manchester University Hospitals

NHS Foundation Trust



Department of Haematology

1st Floor Cobbett House
Manchester Royal Infirmary
Oxford Road, Manchester
M13 9WL

08/06/2011

Project Proposal Professional Doctorate Candidate 381533.

Project Working Title:

Digital Morphology: A National Scheme Supporting Continuous Professional Development.

Consideration of Ethics:

The UK NEQAS scheme for General Haematology has overall governance for the Digital Morphology Scheme. This project was initiated in 2000 and is reviewed by the national Scientific Advisory Panel at the annual meeting of the Morphology Scientific Advisory Group. The overall development of the Digital Morphology scheme is governed by UK NEQAS (H) of which this project forms part. Ethical issues have been considered by both the Morphology SAG and UK NEQAS (H) under two main categories:

a/ The preparation of morphology cases is covered by the policies applied to the collection of all material for EQA purposes in that samples may only be used if they are discard material from routine testing which has been fully anonymised and is not traceable back to a patient. For the use of images in the digital morphology scheme clinical details and patient demographic data are also altered from the original, ie patient ages and test results are altered from the original source data.

b/ The extraction of participant input from the CPD scheme is totally anonymised and not traceable. It is not possible to associate extracted data to any individual. No participant demographic data will be extracted nor made available. This project will use only the morphology features selected and the diagnosis input. Once data is extracted, it will not be possible to associate data with any individual. The participants are not being tested, this data extraction is to interrogate the scheme design and ensure it is meeting objectives.

Yours sincerely



Professor Keith Hyde PhD, FIBMS, FRCPath

Director United Kingdom External Quality Assessment Scheme for
General Haematology [UK NEQAS(H)] &
Director World Health Organisation (WHO)
Collaborating Centre for Quality in Haematology

**Clinical Director (Joint) Greater Manchester Laboratory Medicine
(Pathology) Network**

Consultant Clinical Scientist

Manchester Royal Infirmary Medical Centre

APPENDIX C: DM scheme cases from launch April 2008 to March 2014

Case Survey	Case diagnosis	Number of Participants	Prepared by
0801DM	Haemoglobin HbA/ HbBarts	219	J Burthem & M Brereton
0802DM	T-PLL	603	J Burthem & M Brereton
0803DM	PK deficiency	590	J Burthem & M Brereton
0804DM	Acute Myeloid Leukaemia (Therapy related alkylating agent)	563	J Burthem & M Brereton
0805DM	Burns Victim	659	M Brereton & J Burthem
0901DM	APML	649	M Brereton & J Burthem
0902DM	Glandular Fever	732	J Burthem & M Brereton
0903DM	HIV with associated TTP	752	M Brereton & J Burthem
0904DM	CML	862	J Burthem & M Brereton
0905DM	Haemoglobinopathy (Hb SC disease)	1020	J Burthem & M Brereton
0906DM	Acute Myelomonocytic Leukaemia	1088	J Burthem & M Brereton
1001DM	Primary polycythaemia with dimorphic erythrocyte populations	1103	J Burthem & M Brereton
1002DM	Myelofibrosis transforming to acute leukaemia with previous splenectomy	1318	M Brereton & J Burthem
1003DM	Hereditary Pyropoikilocytosis	964	J Burthem, Z Eke & M Brereton
1004DM	Hairy cell leukaemia with previous splenectomy	1050	M Brereton & J Burthem
1101DM	Vitamin B12 deficiency, megaloblastic anaemia	1154	J Burthem & M Brereton
1102DM	Sickle cell disease (HbSS)	1013	J Burthem & M Brereton
1103DM	G6PD	1142	M Brereton & J Burthem
1104DM	Plasma Cell leuk	999	J Burthem & M Brereton
1105DM	MAHA & Leuco- erthyroblastic picture	1237	J Burthem & M Brereton
1201DM	Haemoglobin SC with Acute Myeloid Leukaemia	1011	M Brereton & J Burthem
1202DM	CLL with Autoimmune Haemolysis	1052	M Brereton & J Burthem
1203DM	Hairy Cell Leukaemia	1261	K Patterson & J Burthem
1204DM	ATLL with G6PD deficiency (oxidative haemolysis)	789	M Brereton & J Burthem
1205DM	T-ALL	935	K Patterson & J Burthem

1206DM	Pelger-Huet anomaly	1108	M Brereton & J Burthem
1301DM	AML with multi-lineage myelodysplasia	1025	J Burthem & M Brereton
1302DM	CLL with AIHA	774	M Brereton & J Burthem
1303DM	Myelofibrosis, progression of primary polycythaemia	956	J Burthem & M Brereton
1304DM	APML microgranular variant	975	J Burthem & M Brereton
1305DM	May Hegglin anomaly	923	K Patterson & D Pelling
1306DM	Liver disease with hyposplensim	995	J Burthem and M Brereton
1401DM	RAEB-II	798	K Patterson & D Pelling
1402DM	Glandular Fever	854	K Patterson & J Burthem

Appendix D: Codes for morphology feature selections available to participants

Table Code List for Morphology Feature Selections available to participants.

WBC		RBC	
code	White Cell Features	Rcode	Red Cell Features
101	Agranulocytosis	301	Acanthocytosis
102	Atypical Cells Myeloid	302	Anisochromia
103	Auer Rods	303	Anisocytosis
104	Band form Neutrophils, left shift	304	Agglutination
105	Basophilia	305	Echinocytes
106	Blast cells	306	Elliptocytes
107	Cerebriform nuclei	307	Ghost, Hemighost cells
108	Cleft nuclei	308	Howell Jolly Bodies
109	Cytoplasmic vacuolation	309	Hypochromic red cells
110	Dohle bodies	310	Keratocytes
111	Eosinophilia	311	Macrocytes
112	Hairy cells	312	Microcytes
113	Hypersegmented neutrophils, right shift	313	Mixed RBC, Dimorphic
114	Hypogranular, agranular cytoplasm neutrophils	314	Microspherocytes
115	Irregular nuclei Lymphoid	315	Normocytic RBC
116	Leucopenia	316	Nucleated RBC
117	Leucocytosis	317	Ovalocytes
118	Large Granular Lymphoid cells	318	Pappenheimer bodies
119	Lymphocytosis	319	Pencil forms
120	Lymphopenia	320	Poikilocytes
121	Lymphoblasts	321	Polychromatic RBC
122	Lymphocytosis neoplastic appearance	322	Punctate Basophilia
123	Lymphoplasmacytoid cells	323	RBC fragments, schistocytes
124	Monocytosis	324	Rouleaux
125	Monocytopenia	325	Sickle cells
126	Myelocytes	326	Spherocytes
127	Neutropenia	327	Stomatocytes
128	Neutrophilia	328	Target cells
129	Pelger Cells	329	Tear drop poikilocytes
130	Plasma cells	Platelet	
131	Prolymphocytes	code	Platelet Features
132	Promyelocytes	201	Clumping
133	Pyknotic nuclei Lymphoid	202	Grey Platelets
134	Reactive/ plasmacytoid lymphocytes / Turk	203	Macrocytic Platelets
135	Reversed neutrophil, lymphocyte ratio	204	Megakaryocyte Fragments
136	Smear, smudge cells	205	Platelet anisocytosis
137	Toxic granulation	206	Satellitism
		207	Thrombocytosis
		208	Thrombocytopenia
000	Blank no selection made		